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(71) Applicant *(for all designated States except US)*: **WASHINGTON UNIVERSITY** [US/US]; One Brookings Drive, St. Louis, MO 63130 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **RICE, Charles M., III** [US/US]; 7316 Colgate Avenue, University City, MO 63130 (US). **BLIGHT, Keril, J.** [US/US]; 4355 Maryland Avenue, St. Louis, MO 63108 (US).

(74) Agents: **KASTEN, Daniel, S.** et al.; Howell & Haferkamp, L.C., Suite 1400, 7733 Forsyth Blvd., St. Louis, MO 63105-1817 (US).

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(54) Title: HCV VARIANTS

(57) Abstract: HCV variants are described. The variants include polynucleotides comprising non-naturally occurring HCV sequences and HCV variants that have a transfection efficiency and ability to survive subpassage greater than HCV that have wild-type polyprotein coding regions. Expression vectors comprising the above polynucleotides and HCV variants are also described, as are the provision of cells and host cells comprising the expression vectors. Methods for identifying a cell line that is permissive for infection with HCV are also provided, as are vaccines comprising the above polynucleotides in a pharmaceutically acceptable carrier. Additionally, methods for inducing immunoprotection to HCV in a primate are described, as are methods for testing a compound for inhibiting HCV replication.

HCV VARIANTS

Background of the Invention

Reference to Government Grant

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Background of the Invention

(1) Field of the Invention

The invention relates to materials and methodologies relating to the production and use of hepatitis C virus (HCV) variants. More specifically, HCV variants are provided that are useful for diagnostic, therapeutic, vaccines and other uses.

(2) Description of the Related Art

Brief general overview of hepatitis C virus

After the development of diagnostic tests for hepatitis A virus and hepatitis B virus, an additional agent, which could be experimentally transmitted to chimpanzees [Alter et al., *Lancet* 1, 459-463 (1978); Hollinger et al., *Intervirology* 10, 60-68 (1978); Tabor et al., *Lancet* 1, 463-466 (1978)], became recognized as the major cause of transfusion-acquired hepatitis. cDNA clones corresponding to the causative non-A non-B (NANB) hepatitis agent, called hepatitis C virus (HCV), were reported in 1989 [Choo et al., *Science* 244, 359-362 (1989)]. This breakthrough has led to rapid advances in diagnostics, and in our understanding of the epidemiology, pathogenesis and molecular virology of HCV (For review, see Houghton *et al., Curr Stud Hematol Blood Transfus 61*, 1-11 (1994); Houghton (1996), pp. 1035-1058 *in* FIELDS VIROLOGY, Fields et al., Eds., Raven Press, Philadelphia; Major et al., *Hepatology 25*, 1527-1538 (1997); Reed and Rice, pp. 1-37 *in* HEPATITIS C VIRUS, Reesink, Ed., Karger, Basel; Hagedorn and Rice (1999), THE HEPATITIS C VIRUSES,

Springer, Berlin). Evidence of HCV infection is found throughout the world, and the prevalence of HCV-specific antibodies ranges from 0.4-2% in most countries to more than 14% in Egypt [Hibbs *et al., J. Inf. Dis.* **168**, 789-790 (1993)]. Besides transmission via blood or blood products, or less frequently by sexual and congenital routes, sporadic cases, not associated with known risk factors, occur and account for more than 40% of HCV cases [Alter *et al., J. Am. Med. Assoc.* **264**, 2231-2235 (1990); Mast and Alter, *Semin. Virol.* **4**, 273-283 (1993)]. Infections are usually chronic [Alter *et al., N. Eng. J. Med.* **327**, 1899-1905 (1992)], and clinical outcomes range from an inapparent carrier state to acute hepatitis, chronic active hepatitis, and cirrhosis which is strongly associated with the development of hepatocellular carcinoma.

Although interferon (IFN)-α has been shown to be useful for the treatment of a minority of patients with chronic HCV infections [Davis *et al., N. Engl. J. Med.* **321**, 1501-1506 (1989); DiBisceglie *et al., New Engl. J. Med.* **321**, 1506-1510 (1989)] and subunit vaccines show some promise in the chimpanzee model [Choo *et al., Proc. Natl. Acad. Sci. USA* **91**, 1294-1298 (1994)], future efforts are needed to develop more effective therapies and vaccines (See, e.g., Tsambiras et al., 1999, Hepatitis C: Hope on the Horizon, Hepatitis C Symposium of 37th Annual Meeting of the Infectious Diseases Society of America, reviewed at http://www.medscape.com/medscape/cno/1999/IDSA/Story.cfm?story_id=913). The considerable diversity observed among different HCV isolates [for review, see Bukh *et al., Sem. Liver Dis.* **15**, 41-63 (1995); Fanning et al., 2000, *Medscape Gastroenterology 2*:mgi6558.fann], the emergence of genetic variants in chronically infected individuals [Enomoto *et al., J. Hepatol.* **17**, 415-416 (1993); Hijikata *et al., Biochem. Biophys. Res. Comm.* **175**, 220-228 (1991); Kato *et al., Biochem. Biophys. Res. Comm.* **189**, 119-127 (1992); Kato *et al., J. Virol.* **67**, 3923-3930 (1993); Kurosaki *et al., Hepatology* **18**, 1293-1299 (1993); Lesniewski *et al., J. Med. Virol.* **40**, 150-156 (1993); Ogata *et al., Proc. Natl. Acad. Sci. USA* **88**, 3392-3396 (1991); Weiner *et al., Virology* **180**, 842-848 (1991); Weiner *et al., Proc. Natl. Acad. Sci. USA* **89**, 3468-3472 (1992)], and the lack of protective immunity elicited after HCV infection [Farci *et al., Science* **258**, 135-140 (1992); Prince *et al., J. Infect. Dis.* **165**, 438-443 (1992)] present major challenges towards these goals.

Molecular Biology of HCV

Classification. Based on its genome structure and virion properties, HCV has been classified as a separate genus in the flavivirus family, which includes two other genera: the flaviviruses (*e.g.*, yellow fever (YF) virus) and the animal pestiviruses (*e.g.*, bovine viral

diarrhea virus (BVDV) and classical swine fever virus (CSFV)) [Francki *et al., Arch. Virol.* **Suppl. 2**, 223 (1991)]. All members of this family have enveloped virions that contain a positive-strand RNA genome encoding all known virus-specific proteins via translation of a single long open reading frame (ORF).

Structure and physical properties of the virion. Studies on the structure and physical properties of the HCV virion have been hampered by the lack of a cell culture system able to support efficient virus replication and the typically low titers of infectious virus present in serum. The size of infectious virus, based on filtration experiments, is between 30-80 nm [Bradley *et al., Gastroenterology* **88**, 773-779 (1985); He *et al., J. Infect. Dis.* **156**, 636-640 (1987); Yuasa *et al., J. Gen. Virol.* **72**, 2021-2024 (1991)]. Initial measurements of the buoyant density of infectious material in sucrose yielded a range of values, with the majority present in a low density pool of < 1.1 g/ml [Bradley *et al., J. Med. Virol.* **34**, 206-208 (1991)]. Subsequent studies have used RT/PCR to detect HCV-specific RNA as an indirect measure of potentially infectious virus present in sera from chronically infected humans or experimentally infected chimpanzees. From these studies, it has become increasingly clear that considerable heterogeneity exists between different clinical samples, and that many factors can affect the behavior of particles containing HCV RNA [Hijikata *et al., J. Virol.* **67**, 1953-1958 (1993); Thomssen *et al., Med. Microbiol. Immunol.* **181**, 293-300 (1992)]. Such factors include association with immunoglobulins [Hijikata *et al.*, (1993) *supra*] or low density lipoprotein [Thomssen *et al.*, 1992, *supra*; Thomssen *et al., Med. Microbiol. Immunol.* **182**, 329-334 (1993)]. In highly infectious acute phase chimpanzee serum, HCV-specific RNA is usually detected in fractions of low buoyant density (1.03-1.1 g/ml) [Carrick *et al., J. Virol. Meth.* **39**, 279-289 (1992); Hijikata *et al.*, (1993) *supra*]. In other samples, the presence of HCV antibodies and formation of immune complexes correlate with particles of higher density and lower infectivity [Hijikata *et al.*, (1993) *supra*]. Treatment of particles with chloroform, which destroys infectivity [Bradley *et al., J. Infect. Dis.* **148**, 254-265 (1983); Feinstone *et al., Infect. Immun.* **41**, 816-821 (1983)], or with nonionic detergents, produced RNA containing particles of higher density (1.17-1.25 g/ml) believed to represent HCV nucleocapsids [Hijikata *et al.*, (1993) *supra*; Kanto *et al., Hepatology* **19**, 296-302 (1994); Miyamoto *et al., J. Gen Virol.* **73**,715-718 (1992)].

There have been reports of negative-sense HCV-specific RNAs in sera and plasma [see Fong *et al., Journal of Clinical Investigation* **88**:1058-60 (1991)]. However, it seems unlikely that such RNAs are essential components of infectious particles since some sera with high infectivity can have low or undetectable levels of negative-strand RNA [Shimizu *et al., Proc. Natl. Acad. Sci. USA* **90**: 6037-6041 (1993)].

The virion protein composition has not been rigorously determined, but HCV structural proteins include a basic C protein and two membrane glycoproteins, E1 and E2.

HCV replication. Early events in HCV replication are poorly understood. A hepatocyte receptor may be CD81, which binds the E2 envelope glycoprotein (Peleri et al., 1998, *Science 282*:938-41). The association of some HCV particles with beta-lipoprotein and immunoglobulins raises the possibility that these host molecules may modulate virus uptake and tissue tropism.

Studies examining HCV replication have been largely restricted to human patients or experimentally inoculated chimpanzees. In the chimpanzee model, HCV RNA is detected in the serum as early as three days post-inoculation and persists through the peak of serum alanine aminotransferase (ALT) levels (an indicator of liver damage) [Shimizu *et al., Proc. Natl. Acad. Sci. USA* **87**: 6441-6444 (1990)]. The onset of viremia is followed by the appearance of indirect hallmarks of HCV infection of the liver. These include the appearance of a cytoplasmic antigen [Shimizu *et al.,* (1990) *supra*] and ultrastructural changes in hepatocytes such as the formation of microtubular aggregates for which HCV previously was referred to as the chloroform-sensitive "tubule forming agent" or "TFA" [reviewed by Bradley, *Prog. Med. Virol.* **37**: 101-135 (1990)]. As shown by the appearance of viral antigens [Blight *et al., Amer. J. Path.* **143**: 1568-1573 (1993); Hiramatsu *et al., Hepatology* **16**: 306-311 (1992); Krawczynski *et al., Gastroenterology* **103**: 622-629 (1992); Yamada *et al., Digest. Dis. Sci.* **38**: 882-887 (1993)] and the detection of positive and negative sense RNAs [Fong *et al.,* (1991) *supra*; Gunji *et al., Arch. Virol.* **134**: 293-302 (1994); Haruna *et al., J. Hepatol.* **18**: 96-100 (1993); Lamas *et al., J. Hepatol.* **16**: 219-223 (1992); Nouri Aria *et al., J. Clin. Inves.* **91**: 2226-34 (1993); Sherker *et al., J. Med. Virol.* **39**: 91-96 (1993); Takehara *et al., Hepatology* **15**: 387-390 (1992); Tanaka *et al., Liver* **13**: 203-208 (1993)], hepatocytes appear to be a major site of HCV replication, particularly during acute infection [Negro *et al., Proc. Natl. Acad. Sci. USA* **89**: 2247-2251 (1992)]. In later stages of HCV infection the appearance of HCV-specific antibodies, the persistence or resolution of viremia, and the severity of liver disease, vary greatly both in the chimpanzee model and in human patients (Fanning et al., *supra*). Although some liver damage may occur as a direct consequence of HCV infection and cytopathogenicity, the emerging consensus is that host immune responses, in particular virus-specific cytotoxic T lymphocytes, may play a more dominant role in mediating cellular damage.

It has been speculated that HCV may also replicate in extra-hepatic reservoir(s). In some cases, RT/PCR or *in situ* hybridization has shown an association of HCV RNA with peripheral blood mononuclear cells including T-cells, B-cells, and monocytes [reviewed in

Blight and Gowans, *Viral Hepatitis Rev.* 1: 143-155 (1995)]. Such tissue tropism could be relevant to the establishment of chronic infections and might also play a role in the association between HCV infection and certain immunological abnormalities such as mixed cryoglobulinemia [reviewed by Ferri *et al., Eur. J. Clin. Invest.* 23: 399-405 (1993)], glomerulonephritis, and rare non-Hodgkin's B-lymphomas [Ferri *et al.,* (1993) *supra*; Kagawa *et al., Lancet* 341: 316-317 (1993)]. However, the detection of circulating negative strand RNA in serum, the difficulty in obtaining truly strand-specific RT/PCR [Gunji *et al.,* (1994) *supra*], and the low numbers of apparently infected cells have made it difficult to obtain unambiguous evidence for replication in these tissues in vivo.

Genome structure. Full-length or nearly full-length genome sequences of numerous HCV isolates have been reported [see, e.g., Lin *et al., J. Virol.* 68: 5063-5073 (1994a); Okamoto *et al., J. Gen. Virol.* 75: 629-635 (1994); Sakamoto *et al., J. Gen. Virol.* 75: 1761-1768 (1994); Trowbridge et al, *Arch Virol. 143*:501-511 (1998); Chamberlain et al, *J. Gen. Virol. 78*:1341-1347 (1997); and citations within Davis, *Am. J. Med. 27*:21S-26S]. HCV genome RNAs are ~9.6 kilobases (kb) in length (Figure 1) and consist of a 5' nontranslated region (5' NTR), a polyprotein coding region consisting of a single long open reading frame (ORF), and a 3' NTR. The 5' NTR is 341-344 bases long and highly conserved. The length of the long ORF varies slightly among isolates, encoding polyproteins of about 3010 to about 3033 amino acids.

The 3' NTR can be divided into three domains. The first (most 5') domain shows considerable diversity both in composition and length (28-42 bases). Recent work by Yanagi et al. [Proc. Natl. Acad. Sci. USA 96:2291-2295(1999)] demonstrate that this region is not necessary for virus replication. The second domain is consists of a variable length polypyrimidine region of poly(A) (in at least HCV-1, type 1a [Han *et al., Proc. Natl. Acad. Sci. USA* 88:1711-1715 (1991)]) or poly(U-ŪC) (see Chen *et al., Virology* 188:102-113 (1992); Okamoto *et al., J. Gen. Virol.* 72:2697-2704 (1991); Tokita *et al., J. Gen. Virol.* 66:1476-83 (1994)]. The third domain, at the extreme 3' end of the genome, is a highly conserved, novel RNA element of about 98 nucleotides, which is necessary for efficient initiation of viral RNA replication [see, e.g., U.S. Patent No. 5,874,565 and U.S. Patent Application No. 08/811,566 (Now U.S. Patent No.____); Kolykhalov *et al., J. Virol.* 70: 3363-3371 (1996); Tanaka *et al., Biochem. Biophys. Res. Comm.* 215: 744-749 (1996); Tanaka *et al., J. Virol.* 70:3307-12 (1996); Yamada *et al., Virology* 223:255-261 (1996); Cheng et al. *J. Virol. 73*:7044-7049]. This domain and the polypyrimidine regions appear to be critical for infectivity *in vivo* [Yanagi et al., *Proc. Natl. Acad. Sci. USA 96*:2291-2295 (1999)].

Translation and proteolytic processing. The highly conserved 5' NTR sequence contains multiple short AUG-initiated ORFs and shows significant homology with the 5' NTR region of pestiviruses [Bukh *et al., Proc. Natl. Acad. Sci. USA* 89: 4942-4946 (1992); Han *et al.,* (1991) *supra*]. A series of stem-loop structures that interact with host factors are present. These structures interact with host factors to initiate polyprotein synthesis through an internal ribosome entry site (IRES) allowing efficient translation initiation at the first AUG of the long ORF [Honda et al., *J. Virol 73*:4941-4951 (1999); Tang et al., *J. Virol. 73*:2359-2364(1999); Psaridi et al., *FEBS Lett. 453*:49-53 (1999)]. Some of the predicted features of the HCV and pestivirus IRES elements are similar to one another [Brown *et al.,* (1992) *supra*]. The ability of this element to function as an IRES suggests that HCV genome RNAs may lack a 5' cap structure.

The organization and processing of the HCV polyprotein (Figure 1) appears to be most similar to that of the pestiviruses. At least 10 polypeptides have been identified and the order of these cleavage products in the polyprotein is NH2-C-E1-E2-p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B-COOH. As shown in Figure 1, proteolytic processing is mediated by host signal peptidase and two HCV-encoded proteinases, the NS2-3 autoproteinase and the NS3-4A serine proteinase [see Rice, *In* "Fields Virology" (B. N. Fields, D. M. Knipe and P. M. Howley, Eds.), Vol. pp. 931-960. Raven Press, New York (1996); Shimotohno *et al., J. Hepatol.* 22: 87-92 (1995) for reviews]. C is a basic protein that serves as the viral core or capsid protein; E1 and E2 are virion envelope glycoproteins; p7 is a hydrophobic protein of unknown function that is inefficiently cleaved from the E2 glycoprotein [Lin *et al.,* (1994a) *supra*; Mizushima *et al., J. Virol.* 68: 6215-6222 (1994); Selby *et al., Virology* 204: 114-122 (1994)]. NS2-NS5B are nonstructural (NS) proteins which function in viral RNA replication complexes. Their functions have been identified as follows: NS2 is a metalloprotease; NS3 is a protease/helicase that contains motifs characteristic of RNA helicases and that has been shown to possess an RNA-stimulated NTPase activity [Suzich *et al., J. Virol.* 67, 6152-6158 (1993)]; NS4A is a co-factor for NS3; NS4B is of unknown function; NS5A interacts with cellular factors to transcriptionally modulate cellular genes and promote cell growth [Ghosh et al., *J. Biol. Chem. 275*:7184-7188] and provide IFNα resistance; and NS5B is a replicase that contains the GDD motif characteristic of the RNA-dependent RNA polymerases of other positive-strand RNA viruses.

Virion assembly and release. This process has not been examined directly, but the lack of complex glycans, the ER localization of expressed HCV glycoproteins [Dubuisson *et al., J. Virol.* 68: 6147-6160 (1994); Ralston *et al., J. Virol.* 67: 6753-6761 (1993)] and the absence of these proteins on the cell surface [Dubuisson *et al.,* (1994) *supra*; Spaete *et al.,*

Virology **188**: 819-830 (1992)] suggest that initial virion morphogenesis may occur by budding into intracellular vesicles. Thus far, efficient particle formation and release has not been observed in transient expression assays, suggesting that essential viral or host factors are absent or blocked. HCV virion formation and release may be inefficient, since a substantial fraction of the virus remains cell-associated, as found for the pestiviruses. Extracellular HCV particles partially purified from human plasma contain complex N-linked glycans, although these carbohydrate moieties were not shown to be specifically associated with E1 or E2 [Sato *et al., Virology* **196**: 354-357 (1993)]. Complex glycans associated with glycoproteins on released virions would suggest transit through the trans-Golgi and movement of virions through the host secretory pathway. If this is correct, intracellular sequestration of HCV glycoproteins and virion formation might then play a role in the establishment of chronic infections by minimizing immune surveillance and preventing lysis of virus-infected cells via antibody and complement.

Genetic variability. As for all positive-strand RNA viruses, the RNA-dependent RNA polymerase of HCV (NS5B) is believed to lack a 3′-5′ exonuclease proof reading activity for removal of misincorporated bases. Replication is therefore error-prone, leading to a "quasi-species" virus population consisting of a large number of variants [Martell *et al., J. Virol.* **66**: 3225-3229 (1992); Martell *et al., J. Virol.* **68**: 3425-3436 (1994)]. This variability is apparent at multiple levels. First, in a chronically infected individual, changes in the virus population occur over time [Ogata *et al.*, (1991) *supra*; Okamoto *et al., Virology* **190**: 894-899 (1992)]; and these changes may have important consequences for disease. A particularly interesting example is the N-terminal 30 residue segment of the E2 glycoprotein, which exhibits a much higher degree of variability than the rest of the polyprotein [for examples, see Higashi *et al., Virology* **197**, 659-668. 1993; Hijikata *et al.*, (1991) *supra*; Weiner *et al.*, (1991) *supra*]. There is accumulating evidence that this hypervariable region, called hypervariable region 1 (HVR1), perhaps analogous to the V3 domain of HIV-1 gp120, may be under immune selection by circulating HCV-specific antibodies [Kato *et al.*, (1993) *supra*; Taniguchi *et al., Virology* **195**: 297-301 (1993); Weiner *et al.*, (1992) *supra*. In this model, antibodies directed against this portion of E2 may contribute to virus neutralization and thus drive the selection of variants with substitutions that permit escape from neutralization. This plasticity suggests that a specific amino acid sequence in the E2 hypervariable region is not essential for other functions of the protein such as virion attachment, penetration, or assembly. Genetic evolution of HVR1 within the first 4 months of infection has been correlated with the ability of a particular strain of the virus to cause chronic infection [Farci et al., *Science 288*:339-344 (2000)].

Genetic variability may also contribute to the spectrum of different responses observed after IFN-α treatment of chronically infected patients. Diminished serum ALT levels and improved liver histology, which usually correlates with a decrease in the level of circulating HCV RNA, is seen in ~40% of those treated [Greiser-Wilke *et al., J. Gen. Virol.* 72: 2015-2019 (1991)]. After treatment, approximately 70% of the responders relapse. In some cases, after a transient loss of circulating viral RNA, renewed viremia is observed during or after the course of treatment. While this might suggest the existence or generation of IFN-resistant HCV genotypes or variants, further work is needed to determine the relative contributions of virus genotype and host-specific differences in immune response.

Sequence comparisons of different HCV isolates around the world have also revealed enormous genetic diversity [reviewed in Bukh *et al.,* (1995) *supra*]. Because of the lack of biologically relevant serological assays such as cross-neutralization tests, HCV types (designated by numbers), subtypes (designated by letters), and isolates are currently grouped on the basis of nucleotide or amino acid sequence similarity. Worldwide, HCV has been classified into six major genotypes and more than 50 subtypes [Purcell, *Hepatology 26*:11S-14S (1997)]. Those of greatest importance in the U.S. are genotype 1, subtypes 1a and 1b (see below and Bukh *et al.,* (1995) *supra* for a discussion of genotype prevalence and distribution). Amino acid sequence similarity between the most divergent genotypes can be a little as ~50%, depending upon the protein being compared. This diversity has important biological implications, particularly for diagnosis, vaccine design, and therapy.

HCV RNA replication. By analogy with other flaviviruses, replication of the positive-sense HCV virion RNA is thought to occur via a minus-strand intermediate. This strategy can be described briefly as follows: (i) uncoating of the incoming virus particle releases the genomic plus-strand, which is translated to produce a single long polyprotein that is probably processed co- and post-translationally to produce individual structural and nonstructural proteins; (ii) the nonstructural proteins form a replication complex that utilizes the virion RNA as template for the synthesis of minus strands; (iii) these minus strands in turn serve as templates for synthesis of plus strands, which can be used for additional translation of viral protein, minus strand synthesis, or packaging into progeny virions. Very few details about HCV replication process are available, due to the lack of a good experimental system for virus propagation. Detailed analyses of authentic HCV replication and other steps in the viral life cycle would be greatly facilitated by the development of an efficient system for HCV replication in cell culture.

Many attempts have been made to infect cultured cells with serum collected from HCV-infected individuals, and low levels of replication have been reported in a number of

cells types infected by this method, including B-cell [Bertolini *et al., Res. Virol. 144*: 281-285 (1993); Nakajima *et al., J. Virol. 70*: 9925-9 (1996); Valli *et al., Res. Virol. 146*:285-288 (1995)]. T-cell (Kato *et al., Biochem. Biophys. Res. Commun. 206*:863-9 (1996); Mizutani *et al., Biochem. Biophys. Res. Comm. 227*:822-826; Mizutani *et al., J. Virol. 70*: 7219-7223 (1996); Nakajima *et al.,* (1996) *supra*; Shimizu and Yoshikura, *J Virol, 68:* 8406-8408 (1994); Shimizu et al., *Proc. Natl. Acad. Sci USA, 89:* 5477-5481 (1992); Shimizu et al., *Proc. Natl. Acad. Sci. USA, 90:* 6037-6041 (1993)], and hepatocyte [Kato et al., *Jpn. J. Cancer Res., 87*: 787-92 (1996); *Tagawa, J. Gastoenterol. and Hepatol., 10*: 523-527 (1995)] cell lines, as well as peripheral blood monocular cells (PBMCs) [Cribier et al., *J. Gen. Virol., 76:* 2485-2491 (1995)], and primary cultures of human fetal hepatocytes [Carloni et al., *Arch. Virol. Suppl. 8*: 31-39 (1993); Cribier et al., (1995) *supra;* Iacovacci et al., *Res. Virol., 144*: 275-279 (1993)] or hepatocytes from adult chimpanzees [Lanford et al., *Virology 202*: 606-14 (1994)]. HCV replication has also been detected in primary hepatocytes derived from a human HCV patient that were infected with the virus in vivo prior to cultivation [Ito et al., *J. Gen. Virol. 77*: 1043-1054 (1996)] and in the human hepatoma cell line Huh7 following transfection with RNA transcribed in vitro from an HCV-1 cDNA clone [Yoo et al., *J. Virol., 69*: 32-38 (1995)]. The reported observation of replication in cells transfected with RNA derived from the HCV-1 clone was puzzling, since this clone lacks the required terminal 3'NTR sequence downstream of the homopolymer tract (see below), and because a number of unusual observations were reported (see the background section of U.S. Patent Application No. 08/811,566 (Now U.S. Patent No.____)). The most well-characterized cell-culture systems for HCV replication utilize a B-cell line (Daudi) or T-cell lines persistently infected with retroviruses (HPB-Ma or MT-2) [Kato et al., (1995) *supra*; Mizutani et al., *Biochem Biophys Res. Comm., 227*: 822-826 (1996a); Mizutani et al., (1996) *supra;* Nakajima et al., (1996) *supra*; Shimizu and Yoshikura, (1994) *supra*]; Shimizu, Proc. Natl. Acad. Sci. USA, *90*: 6037-6041 (1993)]. HPBMa is infected with an amphotropic murine leukemia virus pseudotype of murine sarcoma virus, while MT-2 is infected with human T-cell lymphotropic virus type I (HTLV-I). Clones (HPBMa10-2 and MT-2C) that support HCV replication more efficiently than the uncloned population have been isolated for the two T-cell lines HPBMa and MT-2 [Mizutani et al. *J. Virol.* (1996) *supra*; Shimizu et al., (1993) *supra*]. However, the maximum levels of RNA replication obtained in these lines or in the Daudi lines after degradation of the input RNA is still only about 5 x 10^4 RNA molecules per 10^6 cells [Mizutani et al., (1996) *supra*; Mizutani et al., (1996) *supra*] or 10^4 RNA molecules per ml of culture medium [Nakajima et al., (1996) *supra*]. Although the level of replication is low, long-term infections of up to 198 days in one system [Mizutani et al., *Biochem. Biophys. Res.*

Comm. 227: 822-826 (1996a)] and more than a year in another system [Nakajima et al., (1996) *supra*] have been documented, and infectious virus production has been demonstrated by serial cell-free or cell-mediated passage of the virus to naive cells.

However, efficient replication of an HCV clone comprising the essential conserved terminal 3' NTR sequence had not been observed until the work described in co-pending application 08/811,566, now U.S. Patent No.______, also reported in Kolykhalov et al., *Science 277*:570 (1997), which describes an infectious clone of an isolate of the H strain (type 1a). HCV clones of other subtypes are now known. See, e.g., Yanagi et al., *Virology 262*:250-263 (1999) and Yanagi et al., *Virology 244*:161-172 (1998). While RNA transcripts of these clones are able to infect chimpanzees, cell cultures with these clones only support replication of the virus poorly if at all.

As described in U.S. Patent Application No. 08/811,566 (Now U.S. Patent No.____) (see, e.g., Figure 2 therein) many variations of a functional clone are possible. These include full length or partial sequences where a foreign gene is inserted. The foreign gene can include, e.g., a reporter gene such as β-galactosidase or luciferase, or a gene encoding a selectable marker such as *neo*, *DHFR*, or *tk*. In a specific example disclosed therein, the *neo* gene is operably linked to an internal ribosome entry site (IRES), in order for infected cells to be selected by neomycin or G418 resistance. In this way, presence of replicating HCV RNA in essentially all surviving cells is assured. Additionally, the HCV polyprotein coding region of these clones can be deficient in some or all of the structural genes C, E1 and E2. Thus, replicons can be created without the production of virions. By combining the structural gene-deficient construct with a selectable marker such as *neo*, an efficiently replicating replicon system can be created that can be used to study HCV replication and for other purposes.

Examples of the replicons disclosed in U.S. Patent Application No. 08/811,566 (Now U.S. Patent No.____) is provided in Lohmann et al., *Science 285*:110-113 (1999). In that work, DNA clones of HCV replicons of genotype 1, subtype 1b were constructed. Features of those replicons that are not wild-type HCV features are: a polyprotein coding region lacking the genes encoding the HCV structural proteins; an EMCV IRES immediately 5' to the polyprotein region; and a *neo* gene immediately 3' to the 5' NTR (and the HCV IRES), where the 5' end of the HCV C protein gene is fused to the 5' end of the *neo* gene. When Huh-7 cells were transfected with RNA transcripts of these clones, 6 to >60 G418-resistant colonies arose per experiment. Although the number of cells treated was not specified, about 10^6 - 10^7 cells are normally treated in experiments of this type. Therefore, it is believed that the transfection efficiency, as measured by G418-resistant colonies/total treated, was less than .01% in those studies.

Controls in the Lohmann et al. work included in-frame deletions of the active site of the NS5B polymerase. Although care was taken to remove template DNA from the control transcripts, several G418-resistant control colonies arose. Still, the number of G418-resistant control colonies that arose was much less than the colonies arising from the cells transfected with the replicons containing the wild-type NS5B.

When the G418-resistant colonies were subpassaged, most could not be maintained. Out of more than 303 G418-resistant colonies from non-control replicon treatments, 9 (<3%) could be subpassaged to establish stable cell lines. Replicons established in infected cell lines were sequenced. Although each replicon had a number of amino acid substitutions, the substitutions were scattered throughout the polyprotein coding region. Therefore, there were no mutations that were consistently in one area of the polyprotein coding region, and it was concluded that the establishment of the nine cell lines was not due to adaptive mutations in those replicons. This contention was experimentally tested by transfection/reconstitution experiments that did not provide evidence for adaptive changes.

Despite the advances described above, more efficient HCV-infected cell systems are needed for the production of concentrated virus stocks, structural analysis of virion components, evaluation of putative antiviral therapies including vaccines and antiviral compounds, and improved analyses of intracellular viral processes, including RNA replication. Thus, there is a need for various types of HCV clones that can be used for any of the above purposes. There is also a need to characterize HCV with respect to regions of the genome that might contribute to more efficient *in vitro* or *in vivo* replication and virion production.

Summary of the Invention

Thus, a primary object of the present invention has been to provide DNA encoding non-naturally occurring HCV that is capable of replication.

A related object of the invention is to provide genomic RNA from the above DNA. Still another object of the invention is to provide attenuated HCV DNA or genomic RNA suitable for vaccine development, which can invade a cell and replicate but cannot propagate infectious virus.

Another object of the invention is to provide *in vitro* and *in vivo* models of HCV infection and RNA replication for testing anti-HCV (or antiviral) drugs, for evaluating drug resistance, and for testing attenuated HCV viral vaccines.

An additional object of the invention is to provide replicating HCV replicons. These replicons do not encode structural proteins but may encode a foreign protein such as a reporter gene or a selectable marker.

Still another object of the invention is to provide adaptive replicons, with increased ability to establish replication in continuous or primary cell lines.

Briefly, therefore, the inventors have succeeded in discovering methods of creating replicating HCV variants, including variants with adaptive mutations in HCV that improve their ability to establish RNA replication in culture to create continuous cell lines. These HCV variants and the cell lines that harbor them are useful for studying replication and other HCV characteristics. The cell lines are also useful for developing vaccines and for testing compounds for antiviral properties.

Thus, in some embodiments, the present invention is directed to a polynucleotide comprising a non-naturally occurring HCV sequence that is capable of productive replication in a host cell, or is capable of being transcribed into a non-naturally occurring HCV sequence that is capable of productive replication in a host cell. The HCV sequence comprises, from 5′ to 3′ on the positive-sense nucleic acid, a functional 5′ non-translated region (5' NTR); one or more protein coding regions, including at least one polyprotein coding region that is capable of replicating HCV RNA; and a functional HCV 3′ non-translated region (3' NTR). In preferred embodiments of these polynucleotides, the 5' NTR is an HCV 5' NTR, the polynucleotide comprises at least one IRES selected from the group consisting of a viral IRES, a cellular IRES, and an artificial IRES, and the polyprotein coding region is an HCV polyprotein coding region.

In certain aspects of these embodiments, the above polynucleotides further comprise an adaptive mutation. The adaptive mutation can be such that the polynucleotide has a transfection efficiency into mammalian cells of greater than 0.01%; more preferably greater than 0.1%; even more preferably, greater than 1%; still more preferably greater than 5%, may be about 6%. The adaptive mutations can be such that the polynucleotide is capable of replication in a non-hepatic cell, for example HeLa cells. The adaptive mutations can also cause the polynucleotide to have attenuated virulence, wherein the HCV is impaired in its ability to cause disease, establish chronic infections, trigger autoimmune responses, and transform cells.

In some embodiments of the above described adaptive mutants, the polyprotein region comprises an NS5A gene that is not a wild-type NS5A gene. Preferably, the NS5A gene comprises a mutation. The mutation is preferably within 50 nucleotides of an ISDR or includes the ISDR; more preferably the mutati9on is within 20 nt of the ISDR, or includes the

ISDR. Examples of these adaptive mutations are those that encode an amino acid sequence change selected from the group consisting of Ser (1179) to Ile, Arg (1164) to Gly, Ala(1174) to Ser, Ser(1172) to Cys, and Ser(1172) to Pro of SEQ ID NO:3. Other adaptive mutations include a deletion of at least a portion of the ISDR, and may comprise the entire ISDR. In a particular embodiment, the adaptive mutation comprises a deletion of nucleotides 5345 to 5485 of SEQ ID NO:6.

In some embodiments of the invention polynucleotides, the HCV polyprotein coding region encodes all HCV structural and nonstructural proteins. In other embodiments, the polyprotein coding region is incapable of making infectious HCV particles, making the HCV variant a replicon. Preferably the inability to make HCV particles is due to a deletion in the structural protein coding region. Some embodiments of these replicons further comprise a foreign gene operably linked to a first IRES and the HCV polyprotein coding region operably linked to a second IRES. Preferably, the replicon comprises a genotype 1 HCV sequence, most preferably subtype 1b. Preferred foreign genes in these replicons are selectable markers or reporter genes. In other preferred replicon embodiments, the first IRES is an HCV IRES, the foreign gene is a *neo* gene, and the second IRES is a EMCV IRES. Examples of the above replicons include SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:22 and SEQ ID NO:25. The above replicons also preferably comprise an adaptive mutation, including any of the adaptive phenotypes previously described, including increased transfection efficiency, replication in a non-hepatic cell including HeLa cells, and attenuated virulence, and further comprising any of the adaptive mutations previously described, such as the various NS5A mutations and deletions previously described.

The polynucleotides of the present invention can be in the form of RNA or DNA. Preferred embodiments of the polynucleotides are SEQ ID NOs:5-13 and 22-25, the complements thereof, and the RNA equivalents of the sequences or their complements. In certain embodiments, the polynucleotides are capable of productive infection in a chimpanzee upon intrahepatic injection.

The present invention is also directed to expression vectors comprising DNA forms of any of the above polynucleotides, operably associated with a promoter. Additionally, the invention is directed to cells comprising the above expression vectors as well as host cells comprising any of the polynucleotides described above. The host cells are preferably mammalian cells, more preferably human cells. The host cells are preferably hepatocytes, T-cells, B-cells, or foreskin fibroblasts; most preferably hepatocytes. Certain adaptive mutants can also replicate in HeLa cells. The host cells can be within a non-human mammal capable

of supporting transfection and replication of the HCV RNA, and infection when the HCV RNA encodes a virus particle. A preferred non-human mammal is a chimpanzee.

In additional embodiments, the present invention is directed to methods for identifying a cell line that is permissive for RNA replication with HCV. The method includes the steps of contacting a cell in tissue culture with an infectious amount of the above-described polynucleotides, and detecting replication of HCV variants in cells of the cell line.

The present invention is also directed to a method for producing a cell line comprising replicating HCV. The method includes the steps of (a) transcribing the above-described expression vector to synthesize HCV RNA; (b) transfecting a cell with the HCV RNA; and (c) culturing the cell.

Additionally, the present invention is directed to a vaccine. The vaccine includes any of the above-described polynucleotides, in a pharmaceutically acceptable carrier. In related embodiments, the present invention is directed to a method of inducing immunoprotection to HCV in a primate. The method includes administering the vaccine to the primate.

In further embodiments, the present invention is directed to a method of testing a compound for inhibiting HCV replication. The method includes the steps of (a) treating the above described host cells with the compound; and (b) evaluating the treated host cell for reduced replication, wherein reduced HCV replication indicates the ability of the compound to inhibit replication.

In additional embodiments, the present invention is directed to a method of testing a compound for inhibiting HCV infection. The method comprises treating a host cell with the compound before, during or after infecting the host cell with any of the invention polynucleotides.

In still other embodiments, the present invention is directed to an HCV variant that has (a) transfection efficiency greater than 0.01%, as determined by replication-dependent neomycin resistance, or (b) greater ability of initial colonies of cells transfected with the variant to survive subpassage than wild-type HCV genotype 1, subtype 1b. The HCV variant also has, from 5′ to 3′ on the positive-sense nucleic acid, a functional HCV 5′ non-translated region (5'NTR) comprising an extreme 5'-terminal conserved sequence; an HCV polyprotein coding region; and a functional HCV 3′ non-translated region (3'NTR) comprising a variable region, a polypyrimidine region, and an extreme 3'-terminal conserved sequence. In preferred embodiments, the transfection efficiency is greater than 0.1%; in more preferred embodiments, greater than 1%; in still more preferred embodiments, greater than 5%. In the most preferred embodiments, the transfection efficiency is about 6%.

The variants can have any of the characteristics of the polynucleotides described above. However, preferred variants comprise the NS5A mutation or deletion described for the polynucleotides above.

Among the several advantages achieved by the present invention are the provision of polynucleotides comprising non-naturally occurring HCV sequences; the provision of HCV variants that have a transfection efficiency and ability to survive subpassage greater than HCV forms that have wild-type polyprotein coding regions; the provision of expression vectors comprising the above polynucleotides and HCV variants; the provision of cells and host cells comprising the above expression vectors, the provision of methods for identifying a cell line that is permissive for RNA replication with HCV; the provision of vaccines comprising the above polynucleotides in a pharmaceutically acceptable carrier; the provision of methods for inducing immunoprotection to HCV in a primate; and the provision of methods for testing a compound for inhibiting HCV replication.

Brief Description of the Drawings

FIGURE 1. *HCV genome structure, polyprotein processing, and protein features.* At the top is depicted the viral genome with the structural and nonstructural protein coding regions, and the 5′and 3′ NTRs, and the putative 3′ secondary structure. Boxes below the genome indicate proteins generated by the proteolytic processing cascade. Putative structural proteins are indicated by shaded boxes and the nonstructural proteins by open boxes. Contiguous stretches of uncharged amino acids are shown by black bars. Asterisks denote proteins with N-linked glycans but do not necessarily indicate the position or number of sites utilized. Cleavage sites shown are for host signalase (♦), the NS2-3 proteinase (curved arrow), an the NS3-4A serine protease (⇓).

FIGURE 2. *Strategies for expression of heterologous RNAs and proteins using HCV vectors.* At the top is a diagram of the positive-polarity RNA virus HCV, which expresses mature viral proteins by translation of a single long ORF and proteolytic processing. The regions of the polyprotein encoding the structural proteins (STRUCTURAL) and the nonstructural proteins (REPLICASE) are indicated as lightly-shaded and open boxes, respectively. Below are shown a number of proposed replication-competent "replicon" expression constructs. The first four constructs (A-D) lack structural genes and would therefore require a helper system to enable packaging into infectious virions. Constructs E-G would not require helper functions for replication or packaging. Darkly shaded boxes indicate heterologous or foreign gene sequences (FG). Translation initiation (aug) and termination signals (trn) are indicated

by open triangles and solid diamonds, respectively. Internal ribosomes entry sites (IRES) are shown as boxes with vertical stripes. Constructs A and H illustrate the expression of a heterologous product as an in-frame fusion with the HCV polyprotein. Such protein fusion junctions can be engineered such that processing is mediated either by host or viral proteinases (indicated by the arrow).

FIGURE 3. *Structure of HCVrep1bBartMan.* Two versions of this infectious replicon were constructed as described in Example 1. The first, HCVrep1bBartMan/AvaII, has a *Ava*II restriction site in the variable domain of the 3' NTR that is not present in the 3' NTR of wild-type HCV subtype 1b. The second variant, HCVrep1bBartMan/Δ2U's, has 32, rather than the wild-type 34, U's in the longest stretch of contiguous U's in the polypyrimidine domain of the 3' NTR. The "GDD→AGG" designation shows the inactivating mutation in the non-replicating replicons that were used as polymerase-minus controls in Example 1.

FIGURE 4. *Generation of G418-resistant cell clones.* At the top is a diagram of the HCVrep1bBartMan replicons as described in Figure 3. The middle text summarizes the steps used to isolate the adaptive mutants, which are further described in Example 1. The bottom chart summarizes several characteristics of some of the replicons isolated as described in the Example.

FIGURE 5. *Synthesis of HCV-specific RNA and proteins.* Figure 5A illustrates actinomycin D-resistant RNA replication of four adaptive replicons as further described in the Example. Figure 5B illustrates the immunoprecipitation of ^{35}S-labeled HCV-specific proteins of three adaptive replicons as further described in Example 1.

FIGURE 6. *Detection of NS3 in G418-resistant cell clones.* Monolayers of cells transfected with various replicons as indicated were immunostained with an anti-NS3 antibody. Patterns of staining were similar to cells stained from an infected liver.

FIGURE 7. *Nucleotide and amino acid changes in the NS5A coding region of HCV.* Nucleotide and aimino acid changes in a portion of the NS5A coding region of seven adaptive clones are indicated.

FIGURE 8. *G418-resistant colonies generated after electroporation of replicon RNAs into Huh7 cells.* The ability of an adaptive replicon (Replicon I) to establish colonies after

transfection into Huh7 cells (middle) is compared to the original replicon HCVrepBartMan/AvaII (left) and the same adaptive replicon, but with an inactivating mutation in the polymerase gene (right).

FIGURE 9. *Structures of HCV replicons and full-length HCV RNAs.* The adaptive replicon 5'NTR-EMCV has the 5'NTR fused directly to the EMCV IRES upstream of NS3. Another adaptive replicon, HCVrep/NS2-5B has the non-structural protein, NS2, upstream of NS3. A full-length HCV cDNA clone, HCV FL, was assembled. Also, a bicistronic derivative, HCV FL-neo, was assembled where the 5'NTR is fused to the neomycin phosphotransferase gene and the EMCV IRES is upstream of the HCV open reading frame. In both full-length clones, the open reading frame comprises the structural and non-structural regions, from capsid to NS5B. In addition, all of the replicons and full-length HCV RNAs comprise the mutation coding for Ser to Ile substitution at position 1179 of SEQ ID NO:3, in NS5A.

FIGURE 10. *RNA replication of replicons and full-length HCV RNAs.* The HCV replicons and full-length HCV RNAs shown in FIGURE 9 are replication competent.

Detailed Description of the Invention

Definitions

Various terms are used herein, which have the following definitions:

As used herein, "HCV polyprotein coding region" means the portion of a hepatitis C virus that codes for the polyprotein open reading frame (ORF). This ORF may encode proteins that are the same or different than wild-type HCV proteins. The ORF may also encode only some of the functional proteins encoded by a wild-type polyprotein coding region. The proteins encoded therein may also be from different isolates of HCV, and non-HCV proteins may also be encoded therein.

The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human. Preferably, as used herein, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopoeia or other generally recognized pharmacopoeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic

origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water or aqueous solution saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin.

The phrase "therapeutically effective amount" is used herein to mean an amount sufficient to reduce by at least about 15 percent, preferably by at least 50 percent, more preferably by at least 90 percent, and most preferably prevent, a clinically significant deficit in the activity, function and response of the host. Alternatively, a therapeutically effective amount is sufficient to cause an improvement in a clinically significant condition in the host.

The term "adjuvant" refers to a compound or mixture that enhances the immune response to an antigen. An adjuvant can serve as a tissue depot that slowly releases the antigen and also as a lymphoid system activator that non-specifically enhances the immune response (Hood et al., *Immunology, Second Ed.*, 1984, Benjamin/Cummings: Menlo Park, California, p. 384). Often, a primary challenge with an antigen alone, in the absence of an adjuvant, will fail to elicit a humoral or cellular immune response. Adjuvants include, but are not limited to, complete Freund's adjuvant, incomplete Freund's adjuvant, saponin, mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil or hydrocarbon emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (*bacille Calmette-Guerin*) and *Corynebacterium parvum*. Preferably, the adjuvant is pharmaceutically acceptable.

In a specific embodiment, the term "about" or "approximately" means within 20%, preferably within 10%, and more preferably within 5% of a given value or range.

The term "virus infection" as used herein, refers to the usual way that wild-type virus particles become established in host cells. This generally includes binding to the host cell, uptake, delivery to the cytosol or nucleus, and initiation of replication.

The term "transfection" as used herein, refers to the infection of a cell with a polynucleotide. The polynucleotide can be DNA or RNA. A preferred method of transfecting a cell with an HCV polynucleotide is with replication competent RNA. Delivery to permissive cells can be facilitated by electroporation, charged liposomes, high salt, DE dextran, etc. Replication competent RNAs can also be launched in cells after transfection of DNA such as plasmids or DNA viruses that have been appropriately engineered to provide transcription initiation and termination signals. The transfected RNAs can represent full-length genome RNAs capable of initating a complete replication cycle (including production of progeny virus), or they may be defective lacking one or more RNA elements or proteins essential for virion production but not RNA replication. The latter RNAs, which are lacking

in the ability to produce a virion, will be referred to generally herein as "replication competent RNAs", "RNA replicons" or "replicons".

As used herein, the term "subpassage" connotes the transfer of a colony from one vessel of media to another vessel of media. Examples of vessels of media include dishes, bottles or test tubes with solid or liquid growth media. Unless otherwise indicated, "subpassage" means the transfer of a colony of HCV-transfected cells from a vessel of media where the newly transfected cells were plated to a vessel of media where the colony is isolated.

The term "authentic" is used herein to refer to an HCV polynucleotide, whether a DNA or RNA, that provides for replication and production of functional HCV proteins, or components thereof. The authentic HCV polynucleotides of the present invention are capable of replication and may be infectious, *e.g.*, in a chimpanzee model or in tissue culture, to form viral particles (*i.e.*, "virions"). An authentic HCV polynucleotide of the present invention may also be a "replicon", such that it is incapable of producing the full complement of structural proteins to make a replication competent infectious virion. However, such replicons are capable of RNA replication. Thus, the authentic HCV polynucleotides exemplified in the present application contains all of the virus-encoded information, whether in RNA elements or encoded proteins, necessary for initiation of an HCV RNA replication cycle. The authentic HCV polynucleotides of the invention include modifications described herein, *e.g.*, by site-directed mutagenesis or by culture adaptation, producing a defective or attenuated derivative, or an adaptive variant. Alternatively, sequences from other genotypes or isolates can be substituted for the homologous sequence of the specific embodiments described herein. For example, an authentic HCV nucleic acid of the invention may comprise the adaptive mutations disclosed herein, *e.g.*, on a recipient plasmid, engineered into the polyprotein coding region of a functional clône from another isolate or genotype (either a consensus region or one obtained by very high fidelity cloning). In addition, the HCV polynucleotide of the present invention can include a foreign gene, such as a gene encoding a selectable marker or a reporter protein.

General Description

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell culture, molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Ausubel et al. (ed.) (1993) "Current protocols in molecular biology. Green Publishing Associates, New York; Ausubel et al. (1995), "Short Protocols in Molecular

Biology", John Wiley and Sons; Joseph Sambrook et al. (1989), "Molecular Cloning, A Laboratory Manual", second ed., Cold Spring Harbor Laboratory Press; the series, METHODS IN ENZYMOLOGY (Academic Press, Inc.); *Animal Cell Culture* [R.I. Freshney, ed. (1986)]; Lau, ed. (1999), HEPATITIS C PROTOCOLS, Humana Press, New York; and *Immobilized Cells And Enzymes* [IRL Press, (1986)]; all of which are incorporated by reference.

The present invention is directed to variants of hepatitis C virus (HCV) and methods for producing the variants. As used herein, an HCV variant is a non-naturally occurring HCV sequence that is capable of productive replication in a host cell. The genetic sequence of these variants may comprise insertions, deletions, or base mutations from wild type HCV sequences. As further discussed *infra*, the variants may be produced by genetic engineering, by methods known to the skilled artisan (see, e.g., U.S. Patent Application No. 08/811,566 (Now U.S. Patent No.____); Lohmann et al., *Science 285*:110-113(1999)). Alternatively, as further discussed *infra*, the variants may also be produced by culture selection methods, or a combination of culture selection and genetic engineering.

The variants are in the form of DNA or RNA and can be incorporated into any useful form of those compounds, for example in extrachromosomal DNA that replicates in a microorganism such as *E. coli* or yeast. Included among these are plasmids, phage, BACs, YACs, etc. RNA and virions comprising the variant are also envisioned as within the scope of the invention. The variants of the present invention can also be in the form of cassettes for insertion into a DNA cloning vector. The HCV RNAs are envisioned to be complementary to any HCV DNA disclosed herein. An infectious HCV RNA is a positive strand RNA created from the negative strand template of the HCV DNA clone of the invention.

The variants of the present invention are not narrowly limited to any particular virus subtype. Thus, any particular component of the variant, or the entire variant, may be from any HCV subtype. Preferred subtypes are 1a and 1b, due to the widespread occurrence, as well as the large amount of knowledge available for those two subtypes. However, the use of any other genotype or subtype, as would be considered within the skill of the art, is envisioned as within the scope of the invention. These subtypes include, but are not limited to, any subtypes within genotypes HCV-1, HCV-2, HCV-3, HCV-4, HCV-5, and HCV-6. Moreover, since HCV lacks proofreading activity, the virus itself readily mutates, forming mutant "quasi-species" of HCV that are also contemplated as useful for the present invention. Such mutations are easily identified by sequencing isolates from a subject, as detailed herein or in U.S. Patent Application No. 08/811,566 (Now U.S. Patent No.____). It would be expected that the methods and compositions disclosed herein are useful for any known

subtype or quasi-species, or any subtype or quasi-species not now known but that is discovered in the future.

The HCV variants of the invention include a 5'-NTR conserved sequence, which generally comprises the 5'-terminal sequence GCCAGCC, and which may have additional bases upstream of this conserved sequence without affecting functional activity of the HCV nucleic acid. In a preferred embodiment, the 5'-GCCAGCC includes from 0 to about 10 additional upstream bases; more preferably it includes from 0 to about 5 upstream bases; more preferably still it includes 0, one, or two upstream bases. In specific embodiments, the extreme 5'-terminal sequence may be GCCAGCC; GGCCAGCC; UGCCAGCC; AGCCAGCC; AAGCCAGCC; GAGCCAGCC; GUGCCAGCC; or GCGCCAGCC, wherein the sequence GCCAGCC is the 5'-terminus of SEQ ID NO:1. However, the scope of the HCV variants of the invention encompasses any functional HCV 5' NTR, whether now known or later discovered.

The HCV variants of the invention also include a 3' NTR that comprises a poly-pyrimidine region as is known in wild-type HCV. These polypyrimidine regions are known to comprise, on the positive-strand HCV RNA, a poly(U)/poly(UC) tract or a poly(A) tract. However, the polypyrimidine region of the present invention may also include other polypyrimidine tracts that are not now known but are later found to be functional in infectious HCV. As is known in the art, the polypyrimidine tract may be of variable length: both short (about 75 bases) and long (133 bases) are effective, although an HCV clone containing a long poly(U/UC) tract is found to be highly infectious. Longer tracts may be found in naturally occurring HCV isolates. Thus, an authentic HCV nucleic acid of the invention may have a variable length polypyrimidine tract.

The 3' NTR also comprises, at its extreme 3' end, the highly conserved RNA element of about 98 nucleotides known in the art, and as described in, e.g., U.S. Patent No. 5,874,565, U.S. Patent Application No. 08/811,566 (Now U.S. Patent No.____), and U.S. Patent No. 5,837,463. In a specific aspect, the 3'-NTR extreme terminus is RNA homologous to a DNA having the sequence
5'-TGGTGGCTCCATCTTAGCCCTAGTCACGGCTAGCTGTGAAAGGTCCGTGAGCC GCATGACTGCAGAGAGTGCTGATACTGGCCTCTCTGCTGATCATGT-3' (SEQ ID NO:2). However, the scope of the invention is meant to encompass HCV variants with any HCV 3' NTR that allows virus replication, whether the sequence is now known or later discovered. Included are 3' NTRs that do not comprise a variable region.

The HCV variants of the present invention also include a polyprotein coding region sufficient to allow replication of the HCV RNA. Thus, the polyprotein coding region may be

deficient in functional genes encoding the full complement of the HCV structural genes C, E1 and E2. In addition, the polyprotein coding region may comprise deletions, insertions, or mutations that do not occur in wild-type HCV strains. Further, the polyprotein coding region may be chimeric, such that some of the genes encoded therein are from analogous regions of another virus, as discussed *infra.*

The HCV variants encompassed by the present invention include variants that do not produce virus particles. These variants, which may be termed "replicons", lack the ability to produce a fully functional complement of the structural proteins C, E1 and E2. The inability to produce the functional structural protein component of the HCV virus may be conferred by deletion of the genes encoding one, two, or all three of these proteins. Alternatively, a deletion of a small portion of the coding sequence of one of the structural proteins, or a mutation in a critical region of the coding sequence, or an insertion into the coding sequence could lead to an HCV that cannot produce virions. In the latter case, the insertion can be any sequence that disrupts the ability of the structural protein from becoming part of a virion, and can include functional sequences, such as those that encode a reporter gene (such as β-galactosidase) or those that confers selectability to the cell harboring the replicon (such as *neo*). The above manipulations are entirely within the skill of the art. See, e.g., Lohmann et al., *súpra* and Example 1. As discussed *infra*, such variants are useful for studying replication of the HCV virus, among other things.

The variants of the present invention can also comprise an alteration in the coding sequence of the polyprotein coding region that does not affect the production of functional virions or replicons. These alterations can be such that the amino acid sequence of the mature protein is not changed from the wild-type sequence, due to the degeneracy of the genetic code. Such alterations can be useful, e.g., when they introduce or remove a restriction site, such that the size of HCV fragments produced by digestion with a restriction enzyme is altered. This provides a distinguishing characteristic of that variant, which can be used, e.g., to identify a particular infectious isolate in a multiple infection animal model, or to provide convenient sites for subsequent engineering. Any technique for mutagenesis known in the art can be used, including but not limited to *in vitro* site-directed mutagenesis [Hutchinson, C., *et al.*, 1978, J. Biol. Chem. 253:6551; Zoller and Smith, 1984, DNA 3:479-488; Oliphant *et al.*, 1986, Gene 44:177; Hutchinson *et al.*, 1986, Proc. Natl. Acad. Sci. U.S.A. 83:710], use of TAB® linkers (Pharmacia), etc. PCR techniques are preferred for site directed mutagenesis [see Higuchi, 1989, "Using PCR to Engineer DNA", in *PCR Technology: Principles and Applications for DNA Amplification*, H. Erlich, ed., Stockton Press, Chapter 6, pp. 61-70].

Alterations in the polyprotein coding sequence can also introduce conservative amino acid substitutions in the HCV-encoded proteins. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. Conservatively substituted amino acids can be grouped according to the chemical properties of their side chains. For example, one grouping of amino acids includes those amino acids have neutral and hydrophobic side chains (A, V, L, I, P, W, F, and M); another grouping is those amino acids having neutral and polar side chains (G, S, T, Y, C, N, and Q); another grouping is those amino acids having basic side chains (K, R, and H); another grouping is those amino acids having acidic side chains (D and E); another grouping is those amino acids having aliphatic side chains (G, A, V, L, and I); another grouping is those amino acids having aliphatic-hydroxyl side chains (S and T); another grouping is those amino acids having amine-containing side chains (N, Q, K, R, and H); another grouping is those amino acids having aromatic side chains (F, Y, and W); and another grouping is those amino acids having sulfur-containing side chains (C and M). Preferred conservative amino acid substitutions are: R-K; E-D, Y-F, L-M; V-I, and Q-H. Conservative amino acid substitutions, when conferred on the structural proteins, can alter antigenic epitopes, and thus the immune reactivity of the virus. Those substitutions could also alter the function of the non-structural proteins, such that the virus reproduces at a different rate or is altered in its ability to replicate in cell culture or in an organism. See, e.g., Example 1, where replicon IV is adaptive to cell culture conditions due to the conservative amino acid substitution Ser → Cys in the NS5A protein.

Alterations in the polyprotein coding region could also introduce nonconservative amino acid substitutions in one or more of the proteins encoded therein. Nonconservative substitutions would be expected to alter protein function more drastically than conservative substitutions, and would thus be more likely than conservative substitutions to alter phenotypic characteristics of the virus such as replication rate, adaptation to cell culture or *in vivo* culture, and displayed antigenic determinants. Examples are several adaptive mutations in the NS5A coding region described in the , *infra*.

In some embodiments of the invention, the polyprotein coding region has a consensus sequence derived from more than one HCV isolate. For example, an authentic HCV nucleic acid of the invention may comprise a 5' and 3' sequence from any one subtype of the virus and a polyprotein region from any other subtype. Alternatively, only one of the proteins encoded in the polyprotein might be from another viral subtype. In this way, the effect of a particular protein in conferring characteristics of a particular strain (e.g., reduced virulence, increased replication rate etc.) can be studied.

Chimeras with other viruses, such as with bovine viral diarrhea virus, or another flavivirus, are also envisioned. See, e.g., PCT/US99/08850, incorporated herein by reference. In these embodiments, components of the functional clones can be used to construct chimeric viruses for assay of HCV gene functions and inhibitors thereof [Filocamo *et al.*, *J. Virol.* 71: 1417-1427 (1997); Hahm *et al.*, *Virology* 226: 318-326 (1996); Lu and Wimmer, *Proc Natl Acad Sci USA* 93: 1412-7 (1996)]. In one such extension of the invention, functional HCV elements such as the 5′ IRES, proteases, RNA helicase, polymerase, or 3′ NTR are used to create chimeric derivatives of BVDV whose productive replication is dependent on one or more of these HCV elements. Such BVDV/HCV chimeras can then be used to screen for and evaluate antiviral strategies against these functional components.

Chimeras where a gene encoding a structural or nonstructural protein from a closely related virus such as GB virus B replaces the corresponding HCV gene would also be expected to be functional. See, e.g., Butkiewicz et al., 2000, *J. Virol. 74*, 4291-4301.

Other alterations in the polyprotein coding region contemplated by the present invention include deletions or insertions in the sequence. Such alterations may also alter replication rate, adaptation to various growth conditions, or antigenic determinants. A preferred example of a useful deletion includes the 47 amino acid deletion and replacement of Ser 1182 to Asp 1229 of SEQ ID NO:3 with Tyr, which is an adaptive mutation in the NS5A that provides greater transfection efficiency than HCVs with wild-type NS5A. See Example 1.

Insertions into the polyprotein coding region can be of any length and into any area of the region, provided the modified HCV is still able to replicate. Preferably, the insertion is engineered in frame with the rest of the polyprotein coding region, to allow correct translation of the polyprotein region downstream from the insertion.

Insertions into the polyprotein coding region could introduce a gene encoding a heterologous protein. The choice of heterologous protein is not narrowly limited and can include a protein that is therapeutic to the infected host or cell, or a protein that is harvested and purified for another purpose. Particularly useful heterologous genes include those used for detection of the variant (i.e., reporter genes), or for selection of cells having the variant. Nonlimiting examples of reporter genes useful in the present invention include β-galactosidase, β-glucuronidase, firefly or bacterial luciferase, green fluorescent protein (GFP) and humanized derivatives thereof, cell surface markers, and secreted markers. Such products are either assayed directly or may activate the expression or activity of additional reporters. Nonlimiting examples of selectable markers for mammalian cells include, but are not limited

to, the genes encoding dihydrofolate reductase (*DHFR*; methotrexate resistance), thymidine kinase (*tk*; methotrexate resistance), puromycin acetyl transferase (*pac*; puromycin resistance), neomycin resistance (*neo*; resistance to neomycin or G418), mycophenolic acid resistance (*gpt*), hygromycin resistance, blasticidin resistance, and resistance to zeocin. Other selectable markers can be used in different hosts such as yeast (*ura*3, *his*3, *leu*2, *trp*1).

The present invention also encompasses HCV variants that have alterations in the noncoding regions of the virus. For example, the foreign gene discussed above can also be inserted into a noncoding region of the virus, provided the region with the insert continues to be sufficiently functional to allow replication. To provide for translation of a foreign gene inserted into a noncoding region, the foreign gene must be operatively linked to translational start signals, preferably an internal ribosome entry site (IRES) derived from cellular or viral mRNAs [Jang *et al., Enzyme* 44: 292-309 (1991); Macejak and Sarnow, *Nature* 353: 90-94 1991); Molla *et al., Nature* 356: 255-257 (1992)]. In essence, this strategy creates a second cistron in the variant, separate from the polyprotein coding region cistron. A preferred IRES is the encephalomyocarditis virus (EMCV) IRES.

The foreign gene can also be inserted into the 3' NTR or the 5' NTR. In the 3' NTR, the foreign gene/IRES cassette is preferably inserted into the most 5', variable domain. However, insertions are also envisioned for other regions of the 3' NTR, such as at the junction of the variable region and the polypyrimidine region, or within the polypyrimidine region. In the 5' NTR, the foreign gene is preferably inserted into the area just adjacent (3' to) the internal HCV IRES. In these variants, the foreign gene is engineered to be operably linked to the HCV IRES. Where this is the case, it is preferred that the second IRES (e.g., an EMCV IRES) is engineered just 5' to the polyprotein coding region, to be operably linked to that region. See Example and Lohmann et al., *supra*.

Some of the above strategies for functional expression of heterologous genes have been previously described. See Bredenbeek and Rice, (1992) *supra* for review; see, also Figure 2, which is also Figure 2 of U.S. Patent Application No. 08/811,566 (Now U.S. Patent No.____).

Additionally, noncoding region alterations such as mutations, deletions or insertions that do not encode a foreign protein are within the scope of the invention. For example, mutations, deletions of insertions in the variable or polypyrimidine regions of the 3' NTR, including deletions of the entire variable region, or in the 5' NTR region, that create or destroy restriction sites or make the variant otherwise identifiable can be used advantageously to create a "tagged" variant. See, e.g., Example, where a mutation in the variable region of the 3'

NTR created an easily identifiable *Ava*II restriction site, and where a deletion in the polypyrimidine region created another identifiable variant.

The polyprotein coding sequence can comprise mutants with desirable functional adaptations such as adaptive or attenuated variants. These improved variants can be superior in any desired characteristic. Nonlimiting examples of characteristics that can be improved by the present methods include more rapid or more accurate replication in vivo or in culture, improved transfection efficiency, improved ability to establish subpassaged cell lines, ability to infect a host or a host cell line, virulence, and attenuation of disease symptoms.

Such HCV variants may be adaptive, *e.g.*, by selection for propagation in animals or in vitro. See, e.g., Example. Alternatively, the variants can be engineered by design to comprise the functional adaptation. See, e.g., Example, where a deletion was designed that had increased transfection efficiency and ability to be subpassaged to create a stable cell line, supporting persistent HCV replication.

Non-functional HCV clones, *e.g.*, that are incapable of genuine replication, that fail to produce HCV proteins, that do not produce HCV RNA as detected by Northern analysis, or that fail to infect susceptible animals or cell lines *in vitro*, can be corrected using components of the variants of the present invention. By comparing a variant of an authentic HCV nucleic acid sequence of the invention, with the sequence of the non-functional HCV clone, defects in the non-functional clone can be identified and corrected, and the corrected, replicating variant could have characteristics like the variant, such as an adaptive mutation, etc. All of the methods for modifying nucleic acid sequences available to one of skill in the art to effect modifications in the non-functional HCV genome, including but not limited to site-directed mutagenesis, substitution of the functional sequence from an authentic HCV variant for the homologous sequence in the non-functional clone, etc.

Adaptation of HCV for more improved cell culture characteristics. Replication and transfection efficiency and stability of virions and replicons that have wild-type polyprotein replication in cell culture is inefficient. That is, cells transfected with, e.g., RNA transcripts of clones of these strains replicate slowly in culture and the transfected cells are difficult to maintain. Additionally, transfection efficiency is poor. That is, very few cells that are transfected with the RNA replicon are able to support HCV replication. See, e.g., Example 1 and Lohmann et al., *supra*, where less than 0.01% of Huh-7 cells transfected with RNA transcripts of replicons that have a wild-type (genotype 1, subtype 1b) nonstructural polyprotein coding region grew into colonies on the petri dish where the transfectants were plated. Furthermore, a low percentage of colonies that arose from the original plating (<3%)

could be subpassaged onto another dish of media to form an isolated stable cell line supporting HCV replication.

"Transfection efficiency" is defined by determining the percent of cells having replicating HCV RNA that continue to translate proteins encoded by the transfected nucleic acids. The easiest way to measure this is by determining the percentage of cells that exhibit a characteristic conferred by the HCV RNA. See, e.g., Example 1, where replicons comprising a *neo* gene conferred G418 resistance to the transfected cells, and where the cells were G418 resistant after dividing and forming colonies on the dish where the transfected cells were plated. In that example, G418 resistance would not persist sufficiently for colonies to form unless the HCV RNA was able to replicate and partition into the dividing cells while continuing to replicate and translate the *neo* gene to confer G418 resistance. Transfection efficiency is thus replication dependent, in that the transfected HCV must replicate, transcribe, and translate the measured characteristic (here, G418 resistance). In the context of the *neo* selectable marker, this method of determining transfection efficiency is termed "replication-dependent neomycin resistance". This is the preferred way of measuring transfection efficiency because it only measures transcription from HCV that established itself sufficiently to replicate and partition into dividing cells to form a colony.

Another disadvantageous cell culture characteristic of HCV nucleic acid that has wild-type nonstructural polyprotein genes is that only a low percentage of colonies that form after transfection and selection are able to continue to be maintained upon subpassage as continuous cell lines harboring replicating RNA. This was <3% in Lohmann et al., as discussed *supra*.

Disadvantageous characteristics of HCV having wild-type nonstructural polyprotein genes can be reduced by utilizing certain adaptive mutations and deletions in the NS5A coding region or elsewhere as disclosed herein. Preferred mutations comprise alterations in the encoded amino acid sequence in a region of the NS5A that is just 5' to the coding region of the "interferon sensitivity-determining region" (ISDR). Specifically, various mutations within about 50 nucleotides 5' to the ISDR, more preferably within about 20 nucleotides of the ISDR, where the encoded amino acid sequence is altered, have the effect of adapting an HCV to have higher transfection efficiency and increased ability to withstand subpassage to establish a cell line harboring persistent HCV replication. Specific mutations having this effect include Ser to Ile at amino acid 1179 of SEQ ID NO:3 (subtype 1b nonstructural polyprotein region), conferred, for example, by the mutation g to t at position 5336 of SEQ ID NO:6, embodied in SEQ ID NO:8 (nucleotide[nt]) and SEQ ID NO:16 (amino acid[aa]); Arg to Gly at amino acid 1164 of SEQ ID NO:3, conferred, for example, by the mutation from a to

g at position 5289 of SEQ ID NO:6, embodied in SEQ ID NO:9 (nt) and SEQ ID NO:17 (aa); Ala to Ser at amino acid 1174 of SEQ ID NO:3, conferred, for example, by the mutation from g to t at position 5320 of SEQ ID NO:6, embodied in SEQ ID NO:10 (nt) and the NS5A amino acid sequence of SEQ ID NO:19; Ser to Cys at amino acid 1172 of SEQ ID NO:3, conferred, for example, by the mutation c to g at position 5315 of SEQ ID NO:6, embodied in the NS5A gene SEQ ID NO:11 and the NS5A amino acid sequence of SEQ ID NO:20; and Ser to Pro at amino acid 1172 of SEQ ID NO:3, conferred, for example by the mutation t to c at position 5314 of SEQ ID NO:6, embodied in the NS5A gene SEQ ID NO:12 and the NS5A amino acid SEQ ID NO:21. The adaptive effect of these mutations is surprising since this region of HCV is normally conserved among HCV isolates. Additionally, deletions within the ISDR, including deletions of the entire ISDR and various flanking sequences, cause this adaptive effect. Among these deletions is the substitution of the ISDR and flanking sequence comprising amino acids 1182 to 1229 of SEQ ID NO:3 with a tyrosine, conferred, for example, by the deletion of nt 5345-5485 of SEQ ID NO:6, and embodied in SEQ ID NO:7 (nt) and the NS5A amino acid SEQ ID NO:14.

HCV variants comprising mutations adaptive to cell culture may also be attenuated, that is impaired in its ability to cause disease, establish chronic infections, trigger autoimmune responses, and transform cells.

The present invention also discloses methods for selecting for adaptive HCV variants. These methods comprise the use of an HCV virion or preferably a replicon, which further comprises a dominant selectable marker such as a *neo* gene. Cells are transfected with these variants. The transfectants are plated into selection media, such as G418 when the *neo* gene is utilized in the variant. Colonies that arise to exhibit resistance to the selectable marker are subpassaged into fresh selection media. HCV in colonies that withstand subpassage to establish a cell line harboring HCV replication can be isolated and used to transfect additional cells. Any of these colonies that show increased transfection efficiency or other desirable characteristics, such as the ability to withstand subpassage, are adaptive variants, where the adaptive nature of the variant is conferred by at least one mutation or deletion. Selected areas of the HCV in these adaptive variants are sequenced. Preferably, at least the NS5A is sequenced. More preferably, the entire polyprotein coding region is sequenced. Any mutations in these variants can be further evaluated to determine the adaptive nature of the mutations. That evaluation preferably involves recreating the mutation in an otherwise wild-type coding region and determining if the recreated HCV mutant exhibits the adaptive phenotype of the original mutant.

Adaptive mutations could also be manifested, but are not restricted to: (i) altering the tropism of HCV RNA replication; (ii) altering viral products responsible for deleterious effects on host cells; (iii) increasing or decreasing HCV RNA replication efficiency; (iv) increasing or decreasing HCV RNA packaging efficiency and/or assembly and release of HCV particles; (v) altering cell tropism at the level of receptor binding and entry. Thus, the engineered dominant selectable marker, whose expression is dependent upon productive HCV RNA replication, can be used to select for adaptive mutations in either the HCV replication machinery or the transfected host cell, or both. In addition, dominant selectable markers can be used to select for mutations in the HCV replication machinery that allow higher levels of RNA replication or particle formation. In one example, engineered HCV derivatives expressing a mutant form of DHFR can be used to confer resistance to methotrexate (MTX). As a dominant selectable marker, mutant DHFR is inefficient since nearly stoichiometric amounts are required for MTX resistance. By successively increasing concentrations of MTX in the medium, increased quantities of DHFR will be required for continued survival of cells harboring the replicating HCV RNA. This selection scheme, or similar ones based on this concept, can result in the selection of mutations in the HCV RNA replication machinery allowing higher levels of HCV RNA replication and RNA accumulation. Similar selections can be applied for mutations allowing production of higher yields of HCV particles in cell culture or for mutant HCV particles with altered cell tropism. Such selection schemes involve harvesting HCV particles from culture supernatants or after cell disruption and selecting for MTX-resistant transducing particles by reinfection of naive cells.

Methods similar to the above can be used to establish adaptive variants with variations in characteristics such as the increased or decreased ability to cause infection, the ability to cause infection in a host that wild-type strains are unable to infect, or cells of such a host.

The invention also provides host cell lines transfected with any of the HCV DNA (or HCV RNA) as set forth above. Examples of host cells include, but are by no means limited to, the group consisting of a bacterial cell, a yeast cell, an insect cell, and a mammalian cell. Preferably, the host cell is capable of providing for expression of functional HCV RNA replicase, virions or virus particle proteins.

In a related aspect, as briefly described above, the invention provides a vector for gene therapy or a gene vaccine (also termed herein a genetic vaccine), in which a heterologous protein is inserted into the HCV nucleic acid under conditions that permit expression of the heterologous protein. These vaccines can be either DNA or RNA. In particular, the invention provides an infectious hepatitis C virus (HCV) DNA vector

comprising from 5′ to 3′ on the positive-sense DNA, a promoter; an HCV 5′-non-translated region (NTR) containing the extreme 5′-terminal sequence GCCAGCC; an HCV polyprotein coding region comprising a coding region for a heterologous gene; and a 3′ non-translated region (NTR). Preferably, the promoter is selected from the group consisting of bacteriophage T3, T7, and SP6.

In the embodiments of the invention where the functional HCV nucleic acid is DNA, it may further comprise a promoter operatively associated with the 5′ NTR. For example, but not by way of limitation, the promoter may be selected from the group consisting of bacteriophage T7, T3, and SP6. However, any suitable promoter for transcription of HCV genomic RNA corresponding to the HCV DNA can be used, depending on the specific transcription system employed. For example, for nuclear transcription (*e.g.*, in an animal transgenic for HCV), an endogenous or viral promoter, such as CMV, may be used. Additionally, these promoter-driven HCV DNAs can be incorporated into an extrachromosomally replicating DNA such as a plasmid or a phage.

Various uses of the invention variants are envisioned herein. Uses relevant to therapy and vaccine development include: (i) the generation of defined HCV virus stocks to develop *in vitro* and *in vivo* assays for virus neutralization, attachment, penetration and entry; (ii) structure/function studies on HCV proteins and RNA elements and identification of new antiviral targets; (iii) a systematic survey of cell culture systems and conditions to identify those that support wild-type and variant HCV RNA replication and particle release; (iv) production of adaptive HCV variants capable of more efficient replication in cell culture; (v) production of HCV variants with altered tissue or species tropism; (vi) establishment of alternative animal models for inhibitor evaluation including those supporting HCV variant replication; (vii) development of cell-free HCV replication assays; (viii) production of immunogenic HCV particles for vaccination; (ix) engineering of attenuated HCV derivatives as possible vaccine candidates; (x) engineering of attenuated or defective HCV derivatives for expression of heterologous gene products for gene therapy and vaccine applications; (xi) utilization of the HCV glycoproteins for targeted delivery of therapeutic agents to the liver or other cell types with appropriate receptors.

The invention further provides a method for infecting an animal with HCV variants, where the method comprises administering an infectious dose of HCV variant RNA prepared by transcription of infectious HCV variant DNA. The invention extends to a non-human animal infected with HCV variants or transfected with HCV variant RNA or DNA. Similarly, the invention provides a method for propagating infectious HCV variants *in vitro* comprising culturing a cell line contacted with an infectious amount of HCV variant RNA prepared by

transcription of the infectious HCV DNA, as well as an *in vitro* cell line infected with HCV variants. In a specific embodiment, the cell line is a hepatocyte cell line transfected or infected with an HCV variant in which an IRES-antibiotic resistance cassette has been engineered to provide for selection. The variant may also comprise the adaptive mutations described above.

In accordance with the gene therapy (genetic vaccine) embodiment of the invention, also provided is a method for transducing an animal capable of HCV RNA replication with a heterologous gene, comprising administering an amount of an HCV variant RNA prepared by transcription of the HCV variant DNA vector.

In another embodiment, the invention provides a method for producing HCV particle proteins comprising culturing a host expression cell line transfected with an HCV variant of the invention under conditions that permit expression of HCV particle proteins; and isolating HCV particle proteins from the cell culture. In a specific embodiment, such an expression cell line may be a cell selected from the group consisting of a bacterial cell, a yeast cell, an insect cell, and a mammalian cell.

The invention further provides an HCV virion comprising an HCV variant RNA genome. Such virions can be used in an HCV vaccine, preferably after attenuation, *e.g.*, by heat or chemical treatment, or through selection of attenuated variants by the methods described above.

The *in vivo* and *in vitro* HCV variants of the invention permits controlled screening for anti-HCV agents (*i.e.*, drugs for treatment of HCV), as well as for evaluation of drug resistance. An *in vivo* method for screening for agents capable of modulating HCV replication may comprise administering a candidate agent to an animal containing an HCV variant, and testing for an increase or decrease in a level of HCV variant infection, replication or activity compared to a level of HCV variant infection, replication or activity in the animal prior to administration of the candidate agent; wherein a decrease in the level of HCV variant infection, replication or activity compared to the level of HCV variant infection, replication or activity in the animal prior to administration of the candidate agent is indicative of the ability of the agent to inhibit HCV variant infection, replication or activity. Testing for the level of HCV variant infection or replication can involve measuring the viral titer (*e.g.*, RNA levels) in a serum or tissue sample from the animal; testing for the level of HCV variant activity can involve measuring liver enzymes. Alternatively, an *in vitro* method for screening for agents capable of modulating HCV replication can comprise contacting a cell line supporting a replicating HCV variant with a candidate agent; and thereafter testing for an increase or decrease in a level of HCV variant replication or activity compared to a level of HCV variant

replication or activity in a control cell line or in the cell line prior to administration of the candidate agent, wherein a decrease in the level of HCV variant replication or activity compared to the level of HCV variant replication or activity in a control cell line or in the cell line prior to administration of the candidate agent is indicative of the ability of the agent to inhibit HCV variant replication or activity. In a specific embodiment, testing for the level of HCV variant replication *in vitro* may involve measuring the HCV titer, *(e.g.*, RNA levels) in the cell culture; testing for the level of HCV activity *in vitro* may involve measuring HCV replication.

In addition to the specific HCV variant DNA clones and related HCV variant RNAs, the invention is directed to a method for preparing an HCV variant DNA clone that is capable of replication in a host or host cell line, comprising joining from 5′ to 3′ on the positive-sense DNA a promoter; an HCV 5′ non-translated region (NTR) an HCV polyprotein coding region; and a 3′ non-translated region (NTR), where at least one of these regions is not a naturally occurring region. Preferably, the promoter is selected from the group consisting of bacteriophage T7, T3, and SP6. In a specific embodiment, the extreme 5′-terminal sequence is homologous to SEQ ID NO:1, *e.g.*, the 5′-terminal sequence may be selected from the group consisting of GCCAGCC; GGCCAGCC; UGCCAGCC; AGCCAGCC; AAGCCAGCC; GAGCCAGCC; GUGCCAGCC; and GCGCCAGCC, wherein the sequence GCCAGCC is the 5′-terminus of SEQ ID NO:1.

The 3′-NTR poly-U for use in the method of preparing an HCV variant DNA clone may include a long poly-U region. Similarly, the 3′-NTR extreme terminus may be RNA homologous to a DNA having the sequence 5′-TGGTGGCTCCATCTTAGCCCTAGTCACGGCTAGCTGTGAAAGGTCCGTGAGCC GCATGACTGCAGAGAGTGCTGATACTGGCCTCTCTGCTGATCATGT-3′ (SEQ ID NO:2); in a specific embodiment, the 3′-NTR extreme terminus has the foregoing sequence.

Components of functional HCV variant DNA clones. Components of the functional HCV variant DNA described in this invention can be used to develop cell-free, cell culture, and animal-based screening assays for known or newly identified HCV antiviral targets as described *infra*. For each selected target, it is preferred that the HCV variant used has the wild-type form of the target. Examples of known or suspected targets and assays include [see Houghton, *In* "Fields Virology" (B. N. Fields, D. M. Knipe and P. M. Howley, Eds.), Vol. pp. 1035-1058. Raven Press, New York (1996); Rice, (1996) *supra*; Rice *et al., Antiviral Therapy* **1, Suppl. 4**, 11-17 (1997); Shimotohno, *Hepatology* 21,:887-8 (1995) for reviews], but are not limited to, the following:

The highly conserved 5′ NTR, which contains elements essential for translation of the incoming HCV genome RNA, is one target. It is also likely that this sequence, or its complement, contains RNA elements important for RNA replication and/or packaging. Potential therapeutic strategies include: antisense oligonucleotides (*supra*); trans-acting ribozymes (*supra*); RNA decoys; small molecule compounds interfering with the function of this element (these could act by binding to the RNA element itself or to cognate viral or cellular factors required for activity).

Another target is the HCV C (capsid or core) protein, which is highly conserved and is associated with the following functions: RNA binding and specific encapsidation of HCV genome RNA; transcriptional modulation of cellular [Ray *et al.*, *Virus Res.* **37**: 209-220 (1995)] and other viral [Shih *et al.*, *J. Virol.* **69**: 1160-1171 (1995); Shih *et al.*, *J. Virol.* **67**: 5823-5832 (1993)] genes; binding of cellular helicase [You et al., *J. Virol.* *73*:2841-2853 (1999)]; cellular transformation [Ray *et al.*, *J. Virol.* **70**: 4438-4443 (1996a); Ray et al., *J. Biol. Chem.* *272*:10983-10986(1997)]; prevention of apoptosis [Ray *et al.*, *Virol.* **226**: 176-182 (1996b)]; modulation of host immune response through binding to members of the TNF receptor superfamily [Matsumoto *et al.*, *J. Virol.* **71**: 1301-1309 (1997)].

The E1, E2, and perhaps the E2-p7 glycoproteins that form the components of the virion envelope are targets for potentially neutralizing antibodies. Key steps where intervention can be targeted include: signal peptidase mediated cleavage of these precursors from the polyprotein [Lin *et al.*, (1994a) *supra*]; ER assembly of the E1E2 glycoprotein complex and association of these proteins with cellular chaperones and folding machinery [Dubuisson *et al.*, (1994) *supra*; Dubuisson and Rice, *J. Virol.* **70**: 778-786 (1996)]; assembly of virus particles including interactions between the nucleocapsid and virion envelope; transport and release of virus particles; the association of virus particles with host components such as VLDL [Hijikata *et al.*, (1993) *supra*; Thomssen *et al.*, (1992) *supra*; Thomssen *et al.*, *Med. Microbiol. Immunol.* **182**: 329-334 (1993)] which may play a role in evasion of immune surveillance or in binding and entry of cells expressing the LDL receptor; conserved and variable determinants in the virion which are targets for neutralization by antibodies or which bind to antibodies and facilitate immune-enhanced infection of cells via interaction with cognate Fc receptors; conserved and variable determinants in the virion important for receptor binding and entry; virion determinants participating in entry, fusion with cellular membranes, and uncoating the incoming viral nucleocapsid.

The NS2-3 autoprotease, which is required for cleavage at the 2/3 site is a further target.

The NS3 serine protease and NS4A cofactor which form a complex and mediate four cleavages in the HCV polyprotein [see Rice, (1997) *supra* for review) is yet another suitable target. Targets include the serine protease activity itself; the tetrahedral Zn^{2+} coordination site in the C-terminal domain of the serine protease; the NS3-NS4A cofactor interaction; the membrane association of NS4A; stabilization of NS3 by NS4A; transforming potential of the NS3 protease region [Sakamuro *et al., J Virol* **69**: 3893-6 (1995)].

The NS3 RNA-stimulated NTPase [Suzich *et al.*, (1993) *supra*], RNA helicase [Jin and Peterson, *Arch Biochem Biophys* **323**: 47-53 (1995); Kim *et al., Biochem. Biophys. Res. Commun.* **215**: 160-6 (1995)], and RNA binding [Kanai *et al., FEBS Lett* **376**: 221-4 (1995)] activities; the NS4A protein as a component of the RNA replication complex is another potential target.

The NS5A protein, another replication component, represents another target. This protein is phosphorylated predominantly on serine residues [Tanji *et al., J. Virol.* **69**: 3980-3986 (1995)]. Transcription modulating, cell growth promoting, and apoptosis inhibiting activities of NS5A [Ghosh et al., *J. Biol. Chem. 275*:7184-7188 (2000)] can be targeted. Other characteristics of NS5A that could be targets for therapy include the kinase responsible for NS5A phosphorylation and its interaction with NS5A, and the interaction with NS5A and other components of the HCV replication complex.

The NS5B RNA-dependent RNA polymerase, which is the enzyme responsible for the actual synthesis of HCV positive and negative-strand RNAs, is another target. Specific aspects of its activity include the polymerase activity itself [Behrens *et al., EMBO J.* **15**: 12-22 (1996)]; interactions of NS5B with other replicase components, including the HCV RNAs; steps involved in the initiation of negative- and positive-strand RNA synthesis; phosphorylation of NS5B [Hwang *et al., Virology* **227**:438 (1997)].

Other targets include structural or nonstructural protein functions important for HCV RNA replication and/or modulation of host cell function. Possible hydrophobic protein components capable of forming channels important for viral entry, egress or modulation of host cell gene expression may be targeted.

The 3′ NTR, especially the highly conserved elements (poly (U/UC) tract; 98-base terminal sequence) can be targeted. Therapeutic approaches parallel those described for the 5′ NTR, except that this portion of the genome is likely to play a key role in the initiation of negative-strand synthesis. It may also be involved in other aspects of HCV RNA replication, including translation, RNA stability, or packaging.

The functional HCV variants of the present invention may encode all of the viral proteins and RNA elements required for RNA packaging. These elements can be targeted for

development of antiviral compounds. Electrophoretic mobility shift, UV cross-linking, filter binding, and three-hybrid [SenGupta *et al., Proc. Natl. Acad. Sci. USA* 93: 8496-8501 (1996)] assays can be used to define the protein and RNA elements important for HCV RNA packaging and to establish assays to screen for inhibitors of this process. Such inhibitors might include small molecules or RNA decoys produced by selection *in vitro* [Gold *et al.*, (1995) *supra*].

Complex libraries of the variants of the present invention can be prepared using PCR shuffling, or by incorporating randomized sequences, such as are generated in "peptide display" libraries. Using the "phage method" [Scott and Smith, 1990, *Science 249*:386-390 (1990); Cwirla, et al., *Proc. Natl. Acad. Sci USA., 87*:6378-6382 (1990); Devlin et al., *Science, 249*:404-406 (1990)], very large libraries can be constructed (10^6-10^8 chemical entities). Clones from such libraries can be used to generate other variants or chimeras, e.g., using various HCV subtypes. Such variants can be generated by methods known in the art, without undue experimentation.

A clone that includes a primer and run-off sequence can be used directly for production of functional HCV variant RNA. A large number of vector-host systems known in the art may be used. Examples of vectors include, but are not limited to, *E. coli*, bacteriophages such as lambda derivatives, or plasmids such as pBR322 derivatives or pUC plasmid derivatives, *e.g.*, pGEX vectors, pmal-c, pFLAG, pTET, etc. As is well known, the insertion into a cloning vector can, for example, be accomplished by ligating the DNA fragment into a cloning vector that has complementary cohesive termini. However, if the complementary restriction sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules may be enzymatically modified. Alternatively, any site desired could be produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers may comprise specific chemically synthesized oligonucleotides encoding restriction endonuclease recognition sequences. Recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, etc., so that many copies of the gene sequence are generated.

Expression of HCV RNA and Polypeptides

The HCV variant DNA, which codes for HCV variant RNA and HCV proteins, particularly HCV RNA replicase or virion proteins, can be inserted into an appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. Such elements are termed herein a "promoter." Thus, the HCV variant DNA of the invention is operationally (or operably)

associated with a promoter in an expression vector of the invention. An expression vector also preferably includes a replication origin. The necessary transcriptional and translational signals can be provided on a recombinant expression vector. In a preferred embodiment for *in vitro* synthesis of functional RNAs, the T7, T3, or SP6 promoter is used.

Potential host-vector systems include but are not limited to mammalian cell systems infected with virus recombinant (*e.g.*, vaccinia virus, adenovirus, Sindbis virus, Semliki Forest virus, etc.); insect cell systems infected with recombinant viruses (*e.g.*, baculovirus); microorganisms such as yeast containing yeast vectors; plant cells; or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used.

The cell into which the recombinant vector comprising the HCV variant DNA clone has been introduced is cultured in an appropriate cell culture medium under conditions that provide for expression of HCV RNA or such HCV proteins by the cell. Any of the methods previously described for the insertion of DNA fragments into a cloning vector may be used to construct expression vectors containing a gene consisting of appropriate transcriptional/translational control signals and the protein coding sequences. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombination (genetic recombination).

Expression of HCV variant RNA or protein may be controlled by any promoter/enhancer element known in the art, but these regulatory elements must be functional in the host selected for expression. Promoters which may be used to control expression include, but are not limited to, the SV40 early promoter region (Benoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3′ long terminal repeat of Rous sarcoma virus (Yamamoto, *et al.*, 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner *et al.*, 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster *et al.*, 1982, Nature 296:39-42); prokaryotic expression vectors such as the β-lactamase promoter (Villa-Kamaroff, *et al.*, 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731), or the *tac* promoter (DeBoer, *et al.*, 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25); promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter; and the animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift *et al.*, 1984, Cell 38:639-646; Ornitz *et al.*, 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology

7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl *et al.*, 1984, Cell 38:647-658; Adames *et al.*, 1985, Nature 318:533-538; Alexander *et al.*, 1987, Mol. Cell. Biol. 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder *et al.*, 1986, Cell 45:485-495), albumin gene control region which is active in liver (Pinkert *et al.*, 1987, Genes and Devel. 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf *et al.*, 1985, Mol. Cell. Biol. 5:1639-1648; Hammer *et al.*, 1987, Science 235:53-58), alpha 1-antitrypsin gene control region which is active in the liver (Kelsey *et al.*, 1987, Genes and Devel. 1:161-171), beta-globin gene control region which is active in myeloid cells (Mogram *et al.*, 1985, Nature 315:338-340; Kollias *et al.*, 1986, Cell 46:89-94), myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead *et al.*, 1987, Cell 48:703-712), myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, Nature 314:283-286), and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason *et al.*, 1986, Science 234:1372-1378).

A wide variety of host/expression vector combinations may be employed in expressing the DNA sequences of this invention. Useful expression vectors, for example, may consist of segments of chromosomal, non-chromosomal and synthetic DNA sequences. Suitable vectors include derivatives of SV40 and known bacterial plasmids, *e.g.*, *E. coli* plasmids col El, pCR1, pBR322, pMal-C2, pET, pGEX [Smith *et al.*, 1988, Gene 67:31-40], pMB9 and their derivatives, plasmids such as RP4; phage DNAS, *e.g.*, the numerous derivatives of phage λ, *e.g.*, NM989, and other phage DNA, *e.g.*, M13 and filamentous single stranded phage DNA; yeast plasmids such as the 2μ plasmid or derivatives thereof; vectors useful in eukaryotic cells, such as vectors useful in insect or mammalian cells; vectors derived from combinations of plasmids and phage DNAs, such as plasmids that have been modified to employ phage DNA or other expression control sequences; and the like known in the art.

In addition to the preferred sequencing analysis, expression vectors containing an HCV variant DNA clone of the invention can be identified by four general approaches: (a) PCR amplification of the desired plasmid DNA or specific mRNA, (b) nucleic acid hybridization, (c) presence or absence of selection marker gene functions, (d) analysis with appropriate restriction endonucleases and (e) expression of inserted sequences. In the first approach, the nucleic acids can be amplified by PCR to provide for detection of the amplified product. In the second approach, the presence of nucleic acids in an expression vector can be detected by nucleic acid hybridization using probes comprising sequences that are

homologous to the HCV variant DNA. In the third approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "selection marker" gene functions (*e.g.*, β-galactosidase activity, thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of foreign genes in the vector. In the fourth approach, recombinant expression vectors are identified by digestion with appropriate restriction enzymes. In the fifth approach, recombinant expression vectors can be identified by assaying for the activity, biochemical, or immunological characteristics of the gene product expressed by the recombinant, *e.g.*, HCV RNA, HCV virions, or HCV viral proteins.

For example, in a baculovirus expression systems, both non-fusion transfer vectors, such as but not limited to pVL941 (*Bam*HI cloning site; Summers), pVL1393 (*Bam*HI, *Sma*I, *Xba*I, *Eco*R1, *Not*I, *Xma*III, *Bgl*II, and *Pst*I cloning site; Invitrogen), pVL1392 (*Bgl*II, *Pst*I, *Not*I, *Xma*III, *Eco*RI, *Xba*I, *Sma*I, and *Bam*HI cloning site; Summers and Invitrogen), and pBlue*Bac*III (*Bam*HI, *Bgl*II, *Pst*I, *Nco*I, and *Hind*III cloning site, with blue/white recombinant screening possible; Invitrogen), and fusion transfer vectors, such as but not limited to pAc700 (*Bam*HI and *Kpn*I cloning site, in which the *Bam*HI recognition site begins with the initiation codon; Summers), pAc701 and pAc702 (same as pAc700, with different reading frames), pAc360 (*Bam*HI cloning site 36 base pairs downstream of a polyhedrin initiation codon; Invitrogen(195)), and pBlueBacHisA, B, C (three different reading frames, with *Bam*HI, *Bgl*II, *Pst*I, *Nco*I, and *Hind*III cloning site, an N-terminal peptide for ProBond purification, and blue/white recombinant screening of plaques; Invitrogen) can be used.

Examples of mammalian expression vectors contemplated for use in the invention include vectors with inducible promoters, such as the dihydrofolate reductase (DHFR) promoter, *e.g.*, any expression vector with a *DHFR* expression vector, or a *DHFR*/methotrexate co-amplification vector, such as pED (*Pst*I, *Sal*I, *Sba*I, *Sma*I, and *Eco*RI cloning site, with the vector expressing both the cloned gene and DHFR); [*see* Kaufman, *Current Protocols in Molecular Biology*, 16.12 (1991)]. Alternatively, a glutamine synthetase/methionine sulfoximine co-amplification vector, such as pEE14 (*Hind*III, *Xba*I, *Sma*I, *Sba*I, *Eco*RI, and *Bcl*I cloning site, in which the vector expresses glutamine synthase and the cloned gene; Celltech). In another embodiment, a vector that directs episomal expression under control of Epstein Barr Virus (EBV) can be used, such as pREP4 (*Bam*HI, *Sfi*I, *Xho*I, *Not*I, *Nhe*I, *Hind*III, *Nhe*I, *Pvu*II, and *Kpn*I cloning site, constitutive RSV-LTR promoter, hygromycin selectable marker; Invitrogen), pCEP4 (*Bam*HI, *Sfi*I, *Xho*I, *Not*I, *Nhe*I, *Hind*III, *Nhe*I, *Pvu*II, and *Kpn*I cloning site, constitutive hCMV immediate early gene, hygromycin selectable marker; Invitrogen), pMEP4 (*Kpn*I, *Pvu*I, *Nhe*I, *Hind*III, *Not*I, *Xho*I,

*Sfi*I, *Bam*HI cloning site, inducible methallothionein IIa gene promoter, hygromycin selectable marker: Invitrogen), pREP8 (*Bam*HI, *Xho*I, *Not*I, *Hind*III, *Nhe*I, and *Kpn*I cloning site, RSV-LTR promoter, histidinol selectable marker; Invitrogen), pREP9 (*Kpn*I, *Nhe*I, *Hind*III, *Not*I, *Xho*I, *Sfi*I, and BamHI cloning site, RSV-LTR promoter, G418 selectable marker; Invitrogen), and pEBVHis (RSV-LTR promoter, hygromycin selectable marker, N-terminal peptide purifiable via ProBond resin and cleaved by enterokinase; Invitrogen). Regulatable mammalian expression vectors, can be used, such as Tet and rTet [Gossen and Bujard, *Proc. Natl. Acad. Sci. USA* 89:5547-51 (1992); Gossen *et al.*, *Science* 268:1766-1769 (1995)]. Selectable mammalian expression vectors for use in the invention include pRc/CMV (*Hind*III, *Bst*XI, *Not*I, *Sba*I, and *Apa*I cloning site, G418 selection; Invitrogen), pRc/RSV (*Hind*III, *Spe*I, *Bst*XI, *Not*I, *Xba*I cloning site, G418 selection; Invitrogen), and others. Vaccinia virus mammalian expression vectors [*see*, Kaufman (1991) *supra*] for use according to the invention include but are not limited to pSC11 (*Sma*I cloning site, TK- and β-gal selection), pMJ601 (*Sal*I, *Sma*I, *Afl*I, *Nar*I, *Bsp*MII, *Bam*HI, *Apa*I, *Nhe*I, *Sac*II, *Kpn*I, and *Hind*III cloning site; TK- and β-gal selection), and pTKgptF1S (*Eco*RI, *Pst*I, *Sal*I, *Acc*I, *Hind*II, *Sba*I, *Bam*HI, and Hpa cloning site, TK or XPRT selection).

Examples of yeast expression systems include the non-fusion pYES2 vector (*Xba*I, *Sph*I, *Sho*I, *Not*I, *Gst*XI, *Eco*RI, *Bst*XI, *Bam*HI, *Sac*I, *Kpn*I, and *Hind*III cloning sit; Invitrogen) or the fusion pYESHisA, B, C (*Xba*I, *Sph*I, *Sho*I, *Not*I, *Bst*XI, *Eco*RI, *Bam*HI, *Sac*I, *Kpn*I, and *Hind*III cloning site, N-terminal peptide purified with ProBond resin and cleaved with enterokinase; Invitrogen), to mention just two, can be employed according to the invention.

In addition, a host cell strain may be chosen that modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (*e.g.*, glycosylation, cleavage [*e.g.*, of signal sequence]) of proteins. Expression in yeast can produce a glycosylated product. Expression in eukaryotic cells can increase the likelihood of "native" glycosylation and folding of an HCV protein. Moreover, expression in mammalian cells can provide a tool for reconstituting, or constituting, native HCV virions or virus particle proteins.

A variety of transfection methods, useful for other RNA virus studies, can be utilized herein without undue experimentation. Examples include microinjection, cell fusion, calcium-phosphate cationic liposomes such as lipofectin [Rice *et al.*, *New Biol.* 1:285-296 (1989); see "HCV-based Gene Expression Vectors", *infra*], DE-dextran [Rice *et al.*, *J. Virol.* 61: 3809-3819 (1987)], and electroporation [Bredenbeek *et al.*, *J. Virol.* 67: 6439-6446

(1993); Liljeström *et al., J. Virol.* **65**: 4107-4113 (1991)]. Scrape loading [Kumar *et al., Biochem. Mol. Biol. Int.* **32**: 1059-1066 (1994)] and ballistic methods [Burkholder *et al., J. Immunol. Meth.* **165**: 149-156 (1993)] may also be considered for cell types refractory to transfection by these other methods. A DNA vector transporter may be considered [see, *e.g.*, Wu *et al.*, 1992, J. Biol. Chem. 267:963-967; Wu and Wu, 1988, J. Biol. Chem. 263:14621-14624; Hartmut *et al.*, Canadian Patent Application No. 2,012,311, filed March 15, 1990].

In Vitro Transfection With HCV Variants

Identification of cell lines supporting HCV replication. An important aspect of the invention is a method it provides for developing new and more effective anti-HCV therapy by conferring the ability to evaluate the efficacy of different therapeutic strategies using an authentic and standardized *in vitro* HCV variant replication system. Such assays are invaluable before moving on to trials using rare and valuable experimental animals, such as the chimpanzee, or HCV-infected human patients. The adaptive variants of the invention are particularly useful for this work because their growth in culture and their ability to withstand subpassage is superior to wild-type strains. Also, the replicons disclosed herein are useful because replication can be evaluated without the confounding effects of the structural proteins.

The HCV variant infectious clone technology can also be used to establish *in vitro* and *in vivo* systems for analysis of HCV replication and packaging. These include, but are not restricted to, (i) identification or selection of permissive cell types (for RNA replication, virion assembly and release); (ii) investigation of cell culture parameters (*e.g.*, varying culture conditions, cell activation, etc.) or selection of adaptive mutations that increase the efficiency of HCV replication in cell cultures; and (iii) definition of conditions for efficient production of infectious HCV variant particles (either released into the culture supernatant or obtained after cell disruption). These and other readily apparent extensions of the invention have broad utility for HCV therapeutic, vaccine, and diagnostic development.

General approaches for identifying permissive cell types are outlined below. Optimal methods for RNA transfection (see also, *supra*) vary with cell type and are determined using RNA reporter constructs. These include, for example, the bicistronic replicons disclosed *supra* and in the Examples, and bicistronic virus [Wang *et al., J. Virol.* **67**: 3338-44 (1993)] with the structure 5′-CAT-HCV IRES-LUC-3′. These HCV variants are used both to optimize transfection conditions (using, e.g., by measuring β-galactosidase or CAT [chloramphenicol acetyltransferase] activity to determine transfection efficiency) and to determine if the cell type is permissive for HCV IRES-mediated translation (e.g., by

measuring LUC; luciferase activity). For actual HCV RNA transfection experiments, cotransfection with a 5′ capped luciferase reporter RNA [Wang *et al.*, (1993) *supra*] provides an internal standard for productive transfection and translation. Examples of cell types potentially permissive for HCV replication include, but are not restricted to, primary human cells (*e.g.*, hepatocytes, T-cells, B-cells, foreskin fibroblasts) as well as continuous human cell lines (*e.g.*, HepG2, Huh7, HUT78, HPB-Ma, MT-2, MT-2C, and other HTLV-1 and HTLV-II infected T-cell lines, Namalawa, Daudi, EBV-transformed LCLs). In addition, cell lines of other species, especially those which are readily transfected with RNA and permissive for replication of flaviviruses or pestiviruses (*e.g.*, SW-13, Vero, BHK-21, COS, PK-15, MBCK, etc.), can be tested. Cells are transfected using a method as described *supra*.

For replication assays, RNA transcripts are prepared using the HCV variant and the corresponding non-functional, *e.g.*, ΔGDD (see Examples) derivative as a negative control, for persistence of HCV RNA and antigen in the absence of productive replication. Template DNA (which complicates later analyses) is removed by repeated cycles of DNaseI treatment and acid phenol extraction followed by purification by either gel electrophoresis or gel filtration, to preferably achieve less than one molecule of amplifiable DNA per 10^9 molecules of transcript RNA. DNA-free RNA transcripts are mixed with LUC reporter RNA and used to transfect cell cultures using optimal conditions determined above. After recovery of the cells, RNaseA is added to the media to digest excess input RNA and the cultures incubated for various periods of time. An early timepoint (~1 day post-transfection) will be harvested and analyzed for LUC activity (to verify productive transfection) and positive-strand RNA levels in the cells and supernatant (as a baseline). Samples are collected periodically for 2-3 weeks and assayed for positive-strand RNA levels by QC-RT/PCR [*see* Kolykhalov *et al.*, (1996) *supra*]. Cell types showing a clear and reproducible difference between the intact infectious transcript and the non-functional derivative, *e.g.*, ΔGDD deletion, control can be subjected to more thorough analyses to verify authentic replication. Such assays include measurement of negative-sense HCV RNA accumulation by QC-RT/PCR [Gunji *et al.*, (1994) *supra*; Lanford *et al.*, *Virology* **202**: 606-14 (1994)], Northern-blot hybridization, or metabolic labeling [Yoo *et al.*, (1995) *supra*] and single cell methods, such as *in situ* hybridization [ISH; Gowans *et al.*, *In* "Nucleic Acid Probes" (R. H. Symons, Eds.), Vol. pp. 139-158. CRC Press, Boca Raton. (1989)], *in situ* PCR [followed by ISH to detect only HCV-specific amplification products; Haase *et al.*, *Proc. Natl. Acad. Sci. USA* **87**: 4971-4975 (1990)], and immunohistochemistry.

HCV particles for studying virus-receptor interactions. In combination with the identification of cell lines that are permissive for HCV replication, defined HCV variant

stocks can be used to evaluate the interaction of the HCV with cellular receptors. Assays can be set up which measure binding of the virus to susceptible cells or productive infection, and then used to screen for inhibitors of these processes.

Identification of cell lines for characterization of HCV receptors. Cell lines permissive for HCV RNA replication, as assayed by RNA transfection, can be screened for their ability to be infected by the virus using the HCV variants of the present invention. Cell lines permissive for RNA replication but which cannot be infected by the homologous virus may lack one or more host receptors required for HCV binding and entry. Such cells provide valuable tools for (i) functional identification and molecular cloning of HCV receptors and co-receptors; (ii) characterization of virus-receptor interactions; and (iii) developing assays to screen for compounds or biologics (*e.g.*, antibodies, SELEX RNAs [Bartel and Szostak, *In* "RNA-protein interactions" (K. Nagai and I. W. Mattaj, Eds.), Vol. pp. 82-102. IRL Press, Oxford (1995); Gold *et al., Annu. Rev. Biochem.* **64**: 763-797 (1995)], etc.) that inhibit these interactions. Once defined in this manner, these HCV receptors serve not only as therapeutic targets but may also be expressed in transgenic animals rendering them susceptible to HCV infection [Koike *et al., Dev Biol Stand* **78**: 101-7 (1993); Ren and Racaniello, *J Virol* **66**: 296-304 (1992)]. Such transgenic animal models supporting HCV replication and spread have important applications for evaluating anti-HCV drugs.

The ability to manipulate the HCV glycoprotein structure may also be used to create HCV variants with altered receptor specificity. In one example, HCV glycoproteins can be modified to express a heterologous binding domain for a known cell surface receptor. The approach should allow the engineering of HCV derivatives with altered tropism and perhaps extend infection to non-chimeric small animal models.

Alternative approaches for identifying permissive cell lines. As previously discussed, and as exemplified in the Examples, functional HCV variants can be engineered that comprise selectable markers for HCV replication. For instance, genes encoding dominant selectable markers can be expressed as part of the HCV polyprotein, or as separate cistrons located in permissive regions of the HCV RNA genome.

Animal Models for HCV Infection and Replication

In addition to chimpanzees, the present invention permits development of alternative animal models for studying HCV replication and evaluating novel therapeutics. Using clones of the authentic HCV variants described in this invention as starting material, multiple approaches can be envisioned for establishing alternative animal models for HCV replication. In one manifestation, the variants could be used to inoculate immunodeficient mice harboring

human tissues capable of supporting HCV replication. An example of this art is the SCID:Hu mouse, where mice with a severe combined immunodeficiency are engrafted with various human (or chimpanzee) tissues, which could include, but are not limited to, fetal liver, adult liver, spleen, or peripheral blood mononuclear cells. Besides SCID mice, normal irradiated mice can serve as recipients for engraftment of human or chimpanzee tissues. These chimeric animals would then be substrates for HCV replication after either *ex vivo* or *in vivo* infection with defined virus-containing inocula.

In another manifestation, adaptive mutations allowing HCV replication in alternative species may produce variants that are permissive for replication in these animals. For instance, adaptation of HCV for replication and spread in either continuous rodent cell lines or primary tissues (such as hepatocytes) could enable the virus to replicate in small rodent models. Alternatively, complex libraries of HCV variants created by DNA shuffling [Stemmer, *Proc. Natl. Acad. Sci. USA* 91:10747 (1994)] or other methods known in the art can be created and used for inoculation of potentially susceptible animals. Such animals could be either immunocompetent or immunodeficient, as described above.

The functional activity of HCV variants can be evaluated transgenically. In this respect, a transgenic mouse model can be used [*see, e.g.*, Wilmut *et al.*, *Experientia* 47:905 (1991)]. The HCV RNA or DNA clone can be used to prepare transgenic vectors, including viral vectors, plasmid or cosmid clones (or phage clones). Cosmids may be introduced into transgenic mice using published procedures [Jaenisch, *Science*, 240:1468-1474 (1988)]. In the preparation of transgenic mice, embryonic stem cells are obtained from blastocyst embryos [Joyner, *In Gene Targeting: A Practical Approach. The Practical Approach Series*, Rickwood, D., and Hames, B. D., Eds., IRL Press: Oxford (1993)] and transfected with HCV variant DNA or RNA. Transfected cells are injected into early embryos, *e.g.*, mouse embryos, as described [Hammer *et al., Nature* 315:680 (1985); Joyner, *supra*]. Various techniques for preparation of transgenic animals have been described [U.S. Patent No. 5,530,177, issued June 25, 1996; U.S. Patent No. 5,898,604, issued December 31, 1996]. Of particular interest are transgenic animal models in which the phenotypic or pathogenic effects of a transgene are studied. For example, the effects of a rat phosphoenolpyruvate carboxykinase-bovine growth hormone fusion gene has been studied in pigs [Wieghart *et al.*, *J. Reprod. Fert., Suppl.* 41:89-96 (1996)]. Transgenic mice that express of a gene encoding a human amyloid precursor protein associated with Alzheimer's disease are used to study this disease and other disorders [International Patent Publication WO 96/06927, published March 7, 1996; Quon *et al., Nature* 352:239 (1991)]. Transgenic mice have also been created for the hepatitis delta agent [Polo *et al., J. Virol.* 69:5203 (1995)] and for hepatitis B virus [Chisari,

Curr. Top. Microbiol. Immunol. 206:149 (1996)], and replication occurs in these engineered animals.

Thus, the functional HCV variants described here, or parts thereof, can be used to create transgenic models relevant to HCV replication and pathogenesis. In one example, transgenic animals harboring the entire genome of an HCV variant can be created. Appropriate constructs for transgenic expression of the entire HCV variant genome in a transgenic mouse of the invention could include a nuclear promoter engineered to produce transcripts with the appropriate 5′ terminus, the full-length HCV variant cDNA sequence, a cis-cleaving delta ribozyme [Ball, *J. Virol.* 66: 2335-2345 (1992); Pattnaik *et al.*, *Cell* 69: 1011-1020 (1992)] to produce an authentic 3′ terminus, followed possibly by signals that promote proper nuclear processing and transport to the cytoplasm (where HCV RNA replication occurs). Besides the entire HCV variant genome, animals can be engineered to express individual or various combinations of HCV proteins and RNA elements. For example, animals engineered to express an HCV gene product or reporter gene under the control of the HCV IRES can be used to evaluate therapies directed against this specific RNA target. Similar animal models can be envisioned for most known HCV targets.

Such alternative animal models are useful for (i) studying the effects of different antiviral agents on replication of HCV variants, including replicons, in a whole animal system; (ii) examining potential direct cytotoxic effects of HCV gene products on hepatocytes and other cell types, defining the underlying mechanisms involved, and identifying and testing strategies for therapeutic intervention; and (iii) studying immune-mediated mechanisms of cell and tissue damage relevant to HCV pathogenesis and identifying and testing strategies for interfering with these processes.

Selection and Analysis of Drug-Resistant Variants

Cell lines and animal models supporting HCV replication can be used to examine the emergence of HCV variants with resistance to existing and novel therapeutics. Like all RNA viruses, the HCV replicase is presumed to lack proofreading activity and RNA replication is therefore error prone, giving rise to a high level of variation [Bukh *et al.*, (1995) *supra*]. The variability manifests itself in the infected patient over time and in the considerable diversity observed between different isolates. The emergence of drug-resistant variants is likely to be an important consideration in the design and evaluation of HCV mono and combination therapies. HCV replication systems of the invention can be used to study the emergence of variants under various therapeutic formulations. These might include monotherapy or various combination therapies (*e.g.*, IFN-α, ribavirin, and new antiviral compounds). Resistant

mutants can then be used to define the molecular and structural basis of resistance and to evaluate new therapeutic formulations, or in screening assays for effective anti-HCV drugs (*infra*).

Screening For Anti-HCV Agents

HCV-permissive cell lines or animal models (preferably rodent models) comprising adaptive HCV variants can be used to screen for novel inhibitors or to evaluate candidate anti-HCV therapies. Such therapies include, but would not be limited to, (i) antisense oligonucleotides or ribozymes targeted to conserved HCV RNA targets; (ii) injectable compounds capable of inhibiting HCV replication; and (iii) orally bioavailable compounds capable of inhibiting HCV replication. Targets for such formulations include, but are not restricted to, (i) conserved HCV RNA elements important for RNA replication and RNA packaging; (ii) HCV-encoded enzymes; (iii) protein-protein and protein-RNA interactions important for HCV RNA replication, virus assembly, virus release, viral receptor binding, viral entry, and initiation of viral RNA replication; (iv) virus-host interactions modulating the ability of HCV to establish chronic infections; (v) virus-host interactions modulating the severity of liver damage, including factors affecting apoptosis and hepatotoxicity; (vi) virus-host interactions leading to the development of more severe clinical outcomes including cirrhosis and hepatocellular carcinoma; and (vii) virus-host interactions resulting in other, less frequent, HCV-associated human diseases.

Evaluation of antisense and ribozyme therapies. The present invention extends to the preparation of antisense nucleotides and ribozymes that may be tested for the ability to interfere with HCV replication. This approach utilizes antisense nucleic acid and ribozymes to block translation of a specific mRNA, either by masking that mRNA with an antisense nucleic acid or cleaving it with a ribozyme.

Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule. Reviews of antisense technology include: Baertschi, *Mol. Cell. Endocrinol.* **101**:R15-R24 (1994); Crooke et al., *Annu. Rev. Pharmacol. Toxicol.* 36:107-129 (1996); Alama et al., *Pharmacol. Res.* 36:171-178; and Boyer et al., *J. Hepatol.* **32(1 Suppl)**:98-112(2000). The last review discusses antisense technology as it applies to HCV.

In the cell, they hybridize to that mRNA, forming a double stranded DNA:RNA or RNA:RNA molecule. The cell does not translate an mRNA in this double-stranded form. Therefore, antisense nucleic acids interfere with the expression of mRNA into protein. Oligomers of about fifteen nucleotides and molecules that hybridize to the AUG initiation

codon will be particularly efficient, since they are easy to synthesize and are likely to pose fewer problems than larger molecules when introducing them into organ cells. Antisense methods have been used to inhibit the expression of many genes *in vitro*. Preferably synthetic antisense nucleotides contain phosphoester analogs, such as phosphorothiolates, or thioesters, rather than natural phophoester bonds. Such phosphoester bond analogs are more resistant to degradation, increasing the stability, and therefore the efficacy, of the antisense nucleic acids.

In the genetic antisense approach, expression of the wild-type allele is suppressed because of expression of antisense RNA. This technique has been used to inhibit TK synthesis in tissue culture and to produce phenotypes of the *Kruppel* mutation in *Drosophila*, and the *Shiverer* mutation in mice [Izant *et al., Cell*, 36:1007-1015 (1984); Green *et al., Annu. Rev. Biochem.*, 55:569-597 (1986); Katsuki *et al., Science*, 241:593-595 (1988)]. An important advantage of this approach is that only a small portion of the gene need be expressed for effective inhibition of expression of the entire cognate mRNA. The antisense transgene will be placed under control of its own promoter or another promoter expressed in the correct cell type, and placed upstream of the SV40 polyA site.

Ribozymes are RNA molecules possessing the ability to specifically cleave other single stranded RNA molecules in a manner somewhat analogous to DNA restriction endonucleases. Ribozymes were discovered from the observation that certain mRNAs have the ability to excise their own introns. By modifying the nucleotide sequence of these RNAs, researchers have been able to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it. Recent reviews include Shippy et al., *Mol. Biotechnol.* 12:117-129 (1999); Schmidt, *Mol. Cells* 9:459-463 (1999); Phylactou et al., *Meth. Enzymol.* 313:485-506 (2000); Oketani et al., *J. Hepatol.* 31:628-634 (1999); Macejak et al., *Hepatology* 31:769-776 (2000). The last two references disclose the use of ribozymes for inhibiting HCV. Because they are sequence-specific, only mRNAs with particular sequences are inactivated.

Investigators have identified two types of ribozymes, *Tetrahymena*-type and "hammerhead"-type. *Tetrahymena*-type ribozymes recognize four-base sequences, while "hammerhead"-type recognize eleven- to eighteen-base sequences. The longer the recognition sequence, the more likely it is to occur exclusively in the target mRNA species. Therefore, hammerhead-type ribozymes are preferable to *Tetrahymena*-type ribozymes for inactivating a specific mRNA species, and eighteen base recognition sequences are preferable to shorter recognition sequences.

Screening compound libraries for anti-HCV activity. Various natural product or synthetic libraries can be screened for anti-HCV activity in the *in vitro* or *in vivo* models

comprising HCV variants as provided by the invention. One approach to preparation of a combinatorial library uses primarily chemical methods, of which the Geysen method [Geysen *et al., Molecular Immunology* **23**:709-715 (1986); Geysen *et al.J. Immunologic Method* **102**:259-274 (1987)] and the method of Fodor *et al.*[*Science* **251**:767-773 (1991)] are examples. Furka *et al.*[*14th International Congress of Biochemistry, Volume 5*, Abstract FR:013 (1988); Furka, *Int. J. Peptide Protein Res.* **37**:487-493 (1991)], Houghton [U.S. Patent No. 4,631,211, issued December 1986] and Rutter *et al.*[U.S. Patent No. 5,010,175, issued April 23, 1991] describe methods to produce a mixture of peptides that can be tested for anti-HCV activity.

In another aspect, synthetic libraries [Needels *et al., Proc. Natl. Acad. Sci. USA* **90**:10700-4 (1993); Ohlmeyer *et al., Proc. Natl. Acad. Sci. USA* **90**:10922-10926 (1993); Lam *et al.*, International Patent Publication No. WO 92/00252; Kocis *et al.*, International Patent Publication No. WO 9428028], and the like can be used to screen for anti-HCV compounds according to the present invention. The references describe adaption of the library screening techniques in biological assays.

Defined/engineered HCV variant virus particles for neutralization assays. The variants described herein can be used to produce defined stocks of HCV particles for infectivity and neutralization assays. Homogeneous stocks can be produced in the chimpanzee model, in cell culture systems, or using various heterologous expression systems (*e.g.*, baculovirus, yeast, mammalian cells; see *supra*). These stocks can be used in cell culture or *in vivo* assays to define molecules or gene therapy approaches capable of neutralizing HCV particle production or infectivity. Examples of such molecules include, but are not restricted to, polyclonal antibodies, monoclonal antibodies, artificial antibodies with engineered/optimized specificity, single-chain antibodies (see the section on antibodies, *infra*), nucleic acids or derivatized nucleic acids selected for specific binding and neutralization, small orally bioavailable compounds, etc. Such neutralizing agents, targeted to conserved viral or cellular targets, can be either genotype or isolate-specific or broadly cross-reactive. They could be used either prophylactically or for passive immunotherapy to reduce viral load and perhaps increase the chances of more effective treatment in combination with other antiviral agents (*e.g.*, IFN-α, ribavirin, etc.). Directed manipulation of HCV infectious clones can also be used to produce HCV stocks with defined changes in the glycoprotein hypervariable regions or in other epitopes to study mechanisms of antibody neutralization, CTL recognition, immune escape and immune enhancement. These studies will lead to identification of other virus-specific functions for anti-viral therapy.

Dissection of HCV Replication

Other HCV replication assays. This invention allows directed molecular genetic dissection of HCV replication. Such analyses are expected to (i) validate antiviral targets which are currently being pursued; and (ii) uncover unexpected new aspects of HCV replication amenable to therapeutic intervention. Targets for immediate validation through mutagenesis studies include the following: the 5′ NTR, the HCV polyprotein and cleavage products, and the 3′ NTR. As described above, analyses using the HCV variants and permissive cell cultures can be used to compare parental and mutant replication phenotypes after transfection of cell cultures with infectious RNA. Even though RT-PCR allows sensitive detection of viral RNA accumulation, mutations which decrease the efficiency of RNA replication may be difficult to analyze, unless conditional mutations are recovered. As a complement to first cycle analyses, *trans*-complementation assays can be used to facilitate analysis of HCV mutant phenotypes and inhibitor screening. Chimeric variants comprising portions of heterologous systems (vaccinia, Sindbis, or non-viral) can be used to drive expression of the HCV RNA replicase proteins and/or packaging machinery [see Lemm and Rice, *J. Virol.* 67: 1905-1915 (1993a); Lemm and Rice, *J. Virol.* 67: 1916-1926 (1993b); Lemm *et al., EMBO J.* 13: 2925-2934 (1994); Li *et al., J. Virol.* 65: 6714-6723 (1991)]. If these elements are capable of functioning in *trans*, then co-expression of RNAs with appropriate *cis*-elements should result in RNA replication/packaging. Such systems therefore mimic steps in authentic RNA replication and virion assembly, but uncouple production of viral components from HCV replication. If HCV replication is somehow self-limiting, heterologous systems may drive significantly higher levels of RNA replication or particle production, facilitating analysis of mutant phenotypes and antiviral screening. A third approach is to devise cell-free systems for HCV template-dependent RNA replication. A coupled translation/replication and assembly system has been described for poliovirus in HeLa cells [Barton and Flanegan, *J. Virol.* 67: 822-831 (1993); Molla *et al., Science* 254: 1647-1651 (1991)], and a template-dependent *in vitro* assay for initiation of negative-strand synthesis has been established for Sindbis virus. Similar *in vitro* systems using HCV variants are invaluable for studying many aspects of HCV replication as well as for inhibitor screening and evaluation. An example of each of these strategies follows.

Trans-complementation of HCV RNA replication and/or packaging using viral or non-viral expression systems. Heterologous systems can be used to drive HCV replication. For example, the vaccinia/T7 cytoplasmic expression system has been extremely useful for trans-complementation of RNA virus replicase and packaging functions [see Ball, (1992) *supra*; Lemm and Rice, (1993a) *supra*; Lemm and Rice, (1993b) *supra*; Lemm *et al.*, (1994)

supra; Pattnaik *et al.*, (1992) *supra*; Pattnaik *et al.*, *Virology* 206: 760-4 (1995); Porter *et al.*, *J. Virol.* 69: 1548-1555 (1995)]. In brief, a vaccinia recombinant (vTF7-3) is used to express T7 RNA polymerase (T7RNApol) in the cell type of interest. Target cDNAs, positioned downstream from the T7 promoter, are delivered either as vaccinia recombinants or by plasmid transfection. This system leads to high level RNA and protein expression. A variation of this approach, which obviates the need for vaccinia (which could interfere with HCV RNA replication or virion formation), is the pT7T7 system where the T7 promoter drives expression of T7RNApol [Chen *et al.*, *Nucleic Acids Res.* 22: 2114-2120. (1994)]. pT7T7 is mixed with T7RNApol (the protein) and co-transfected with the T7-driven target plasmid of interest. Added T7RNApol initiates transcription, leading to it own production and high level expression of the target gene. Using either approach, RNA transcripts of variants with precise 5′ and 3′ termini can be produced using the T7 transcription start site (5′) and the cis-cleaving HCV ribozyme (Rz) (3′) [Ball, (1992) *supra*; Pattnaik *et al.*, (1992) *supra*].

These or similar expression systems can be used to establish assays for HCV RNA replication and particle formation using HCV variants, and for evaluation of compounds which might inhibit these processes. T7-driven protein expression constructs and full-length HCV variants incorporating the HCV ribozyme following the 3′ NTR can also be used. A typical experimental plan to validate the assay as described for pT7T7, although essentially similar assays can be envisioned using vTF7-3 or cell lines expressing the T7 RNA polymerase. HCV-permissive cells are co-transfected with pT7T7+T7RNApol+p90/HCVFLlong pU Rz (or a negative control, such as ΔGDD). At different times post-transfection, accumulation of HCV proteins and RNAs, driven by the pT7T7 system, are followed by Western and Northern blotting, respectively. To assay for HCV-specific replicase function, actinomycin D is added to block DNA-dependent T7 transcription [Lemm and Rice, (1993a), *supra*] and actinomycin D-resistant RNA synthesis is monitored by metabolic labeling. Radioactivity will be incorporated into full-length HCV RNAs for p90/HCVFL long pU/Rz, but not for p90/HCVFLΔGDD/Rz. Using HCV variants of the invention, this assay system, or elaborated derivatives, can be used to screen for inhibitors and to study their effects on HCV RNA replication.

Cell-free systems for assaying HCV replication and inhibitors thereof. Cell-free assays for studying HCV RNA replication and inhibitor screening can also be established using the variants described in this invention. Either virion or transcribed RNAs are used as substrate RNA. For HCV, full-length HCV variant RNAs transcribed *in vitro* can be used to program such *in vitro* systems and replication assayed essentially as described for poliovirus

[see Barton *et al.*, (1995) *supra*]. In case hepatocyte-specific or other factors are required for HCV variant RNA replication, the system can be supplemented with hepatocyte or other cell extracts, or alternatively, a comparable system can be established using cell lines which have been shown to be permissive for replication of the HCV variants.

One concern about this approach is that proper cell-free synthesis and processing of the HCV polyprotein must occur. Sufficient quantities of properly processed replicase components may be difficult to produce. To circumvent this problem, the T7 expression system can be used to express high levels of HCV replicase components in appropriate cells [see Lemm *et al.*, (1997) *supra*]. P15 membrane fractions from these cells (with added buffer, Mg^{2+}, an ATP regenerating system, and NTPs) should be able to initiate and synthesize full-length negative-strand RNAs upon addition of HCV-specific template RNAs.

Establishment of either or both of the above assays allows rapid and precise analysis of the effects of HCV mutations, host factors, involved in replication and inhibitors of the various steps in HCV RNA replication. These systems will also establish the requirements for helper systems for preparing replication-deficient HCV vectors.

Vaccination and Protective Immunity

There are still many unknown parameters that impact on development of effective HCV vaccines. It is clear in both man and the chimpanzee that some individuals can clear the infection. Also, 10-20% of those treated with IFN or about twice this percentage treated with IFN and ribavirin show a sustained response as evidenced by lack of circulating HCV RNA. Other studies have shown a lack of protective immunity, as evidenced by successful reinfection with both homologous virus as well as with more distantly related HCV types [Farci *et al.*, (1992) *supra*; Prince *et al.*, (1992) *supra*]. Nonetheless, chimpanzees immunized with subunit vaccines consisting of E1E2 oligomers and vaccinia recombinants expressing these proteins are partially protected against low dose challenges [Choo *et al.*, *Proc. Natl. Acad. Sci. USA* 91:1294 (1994)]. The HCV variant technology described in this invention has utility not only for basic studies aimed at understanding the nature of protective immune responses against HCV, but also for novel vaccine production methods.

Active immunity against HCV can be induced by immunization (vaccination) with an immunogenic amount of an attenuated or inactivated HCV variant virion, or HCV virus particle proteins, preferably with an immunologically effective adjuvant. An "immunologically effective adjuvant" is a material that enhances the immune response.

Selection of an adjuvant depends on the subject to be vaccinated. Preferably, a pharmaceutically acceptable adjuvant is used. For example, a vaccine for a human should

avoid oil or hydrocarbon emulsion adjuvants, including complete and incomplete Freund's adjuvant. One example of an adjuvant suitable for use with humans is alum (alumina gel). A vaccine for an animal, however, may contain adjuvants not appropriate for use with humans.

An alternative to a traditional vaccine comprising an antigen and an adjuvant involves the direct *in vivo* introduction of DNA or RNA encoding the antigen into tissues of a subject for expression of the antigen by the cells of the subject's tissue. Such vaccines are termed herein genetic vaccines, DNA vaccines, genetic vaccination, or nucleic acid-based vaccines. Methods of transfection as described above, such as DNA vectors or vector transporters, can be used for DNA vaccines.

DNA vaccines are described, e.g., in International Patent Publication WO 95/20660 and International Patent Publication WO 93/19183, the disclosures of which are hereby incorporated by reference in their entireties. The ability of directly injected DNA that encodes a viral protein or genome to elicit a protective immune response has been demonstrated in numerous experimental systems [Conry *et al., Cancer Res.*, 54:1164-1168 (1994); Cox *et al., Virol*, 67:5664-5667 (1993); Davis *et al., Hum. Mole. Genet.*, 2:1847-1851 (1993); Sedegah *et al., Proc. Natl. Acad. Sci.*, 91:9866-9870 (1994); Montgomery *et al., DNA Cell Bio.*, 12:777-783 (1993); Ulmer *et al., Science*, 259:1745-1749 (1993); Wang *et al., Proc. Natl. Acad. Sci.*, 90:4156-4160 (1993); Xiang *et al., Virology*, 199:132-140 (1994)]. Studies to assess this strategy in neutralization of influenza virus have used both envelope and internal viral proteins to induce the production of antibodies, but in particular have focused on the viral hemagglutinin protein (HA) [Fynan *et al., DNA Cell. Biol.*, 12:785-789 (1993A); Fynan *et al., Proc. Natl. Acad. Sci.*, 90:11478-11482 (1993B); Robinson *et al., Vaccine*, 11:957, (1993); Webster *et al., Vaccine*, 12:1495-1498 (1994)].

Vaccination through directly injecting DNA or RNA that encodes a protein to elicit a protective immune response produces both cell-mediated and humoral responses. This is analogous to results obtained with live viruses [Raz *et al., Proc. Natl. Acad. Sci.*, 91:9519-9523 (1994); Ulmer, 1993, *supra*; Wang, 1993, *supra;* Xiang, 1994, *supra*]. Studies with ferrets indicate that DNA vaccines against conserved internal viral proteins of influenza, together with surface glycoproteins, are more effective against antigenic variants of influenza virus than are either inactivated or subvirion vaccines [Donnelly *et al., Nat.Medicine*, 6:583-587 (1995)]. Indeed, reproducible immune responses to DNA encoding nucleoprotein have been reported in mice that last essentially for the lifetime of the animal [Yankauckas *et al., DNA Cell Biol.*, 12: 771-776 (1993)].

A vaccine of the invention can be administered via any parenteral route, including but not limited to intramuscular, intraperitoneal, intravenous, intraarterial (*e.g.*, Ripatic artery)

and the like. Preferably, since the desired result of vaccination is to elucidate an immune response to HCV, administration directly, or by targeting or choice of a viral vector, indirectly, to lymphoid tissues, *e.g.*, lymph nodes or spleen. Since immune cells are continually replicating, they are ideal target for retroviral vector-based nucleic acid vaccines, since retroviruses require replicating cells.

Passive immunity can be conferred to an animal subject suspected of suffering an infection with HCV by administering antiserum, neutralizing polyclonal antibodies, or a neutralizing monoclonal antibody against HCV to the patient. Although passive immunity does not confer long-term protection, it can be a valuable tool for the treatment of an acute infection of a subject who has not been vaccinated. Preferably, the antibodies administered for passive immune therapy are autologous antibodies. For example, if the subject is a human, preferably the antibodies are of human origin or have been "humanized," in order to minimize the possibility of an immune response against the antibodies. In addition, genes encoding neutralizing antibodies can be introduced in vectors for expression *in vivo*, *e.g.*, in hepatocytes.

Antibodies for passive immune therapy. Preferably, HCV variant virions or virus particle proteins prepared as described above are used as an immunogen to generate antibodies that recognize HCV. The variants utilized should have wild-type coat Such antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments, and an Fab expression library. Various procedures known in the art may be used for the production of polyclonal antibodies to HCV. For the production of antibody, various host animals can be immunized by injection with the HCV virions or polypeptide, *e.g.*, as describe *infra*, including but not limited to rabbits, mice, rats, sheep, goats, etc. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (*bacille Calmette-Guerin*) and *Corynebacterium parvum*.

For preparation of monoclonal antibodies directed toward HCV as described above, any technique that provides for the production of antibody molecules by continuous cell lines in culture may be used. These include but are not limited to the hybridoma technique originally developed by Kohler and Milstein [*Nature* **256**:495-497 (1975)], as well as the trioma technique, the human B-cell hybridoma technique [Kozbor *et al., Immunology Today* 4:72 1983); Cote *et al., Proc. Natl. Acad. Sci. U.S.A.* **80**:2026-2030 (1983)], and the EBV-

hybridoma technique to produce human monoclonal antibodies [Cole *et al.*, in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96 (1985)]. In an additional embodiment of the invention, monoclonal antibodies can be produced in germ-free animals [International Patent Publication No. WO 89/12690, published 28 December 1989]. In fact, according to the invention, techniques developed for the production of "chimeric antibodies" [Morrison *et al.*, *J. Bacteriol.* 159:870 (1984); Neuberger *et al.*, *Nature* 312:604-608 (1984); Takeda *et al.*, *Nature* 314:452-454 (1985)] by splicing the genes from a mouse antibody molecule specific for HCV together with genes from a human antibody molecule of appropriate biological activity can be used; such antibodies are within the scope of this invention. Such human or humanized chimeric antibodies are preferred for use in therapy of human diseases or disorders (described *infra*), since the human or humanized antibodies are much less likely than xenogenic antibodies to induce an immune response, in particular an allergic response, themselves.

According to the invention, techniques described for the production of single chain antibodies [U.S. Patent Nos. 5,476,786 and 5,132,405 to Huston; U.S. Patent 4,946,778] can be adapted to produce HCV-specific single chain antibodies. An additional embodiment of the invention utilizes the techniques described for the construction of Fab expression libraries [Huse *et al.*, *Science* 246:1275-1281 (1989)] to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

Antibody fragments containing the idiotype of the antibody molecule can be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragment which can be produced by pepsin digestion of the antibody molecule; the Fab′ fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent.

HCV particles for subunit vaccination. The functional HCV variants of the present invention can be used to produce HCV-like particles for vaccination. Proper glycosylation, folding, and assembly of HCV particles may be important for producing appropriately antigenic and protective subunit vaccines. Several methods can be used for particle production. They include engineering of stable cell lines for inducible or constitutive expression of HCV-like particles (using bacterial, yeast or mammalian cells), or the use of higher level eukaryotic heterologous expression systems such as recombinant baculoviruses, vaccinia viruses [Moss, *Proc. Natl. Acad. Sci. U.S.A.* 93: 11341-11348 (1996)], or alphaviruses [Frolov *et al.*, (1996) *supra*]. HCV particles for immunization may be purified from either the media or disrupted cells, depending upon their localization. Such purified

HCV particles or mixtures of particles representing a spectrum of HCV genotypes, can be injected with our without various adjuvants to enhance immunogenicity.

Infectious non-replicating HCV particles. In another manifestation, particles of HCV variants capable of receptor binding, entry, and translation of genome RNA can be produced. Heterologous expression approaches for production of such particles include, but are not restricted to, *E. coli*, yeast, or mammalian cell lines, appropriate host cells infected or harboring recombinant baculoviruses, recombinant vaccinia viruses, recombinant alphaviruses or RNA replicons, or recombinant adenoviruses, engineered to express appropriate HCV RNAs and proteins. In one example, two recombinant baculoviruses are engineered. One baculovirus expresses the HCV structural proteins (*e.g.* C-E1-E2-p7) required for assembly of HCV particles. A second recombinant expresses the entire HCV genome RNA, with precise 5′ and 3′ ends, except that a deletion, such as ΔGDD or GDD→AAG (see example 1), is included to inactivate the HCV NS5B RDRP. Other mutations abolishing productive HCV replication could also be utilized instead or in combination. Cotransfection of appropriate host cells (Sf9, Sf21, etc.) with both recombinants will produce high levels of HCV structural proteins and genome RNA for packaging into HCV-like particles. Such particles can be produced at high levels, purified, and used for vaccination. Once introduced into the vaccinee, such particles will exhibit normal receptor binding and infection of HCV-susceptible cells. Entry will occur and the genome RNA will be translated to produce all of the normal HCV antigens, except that further replication of the genome will be completely blocked given the inactivated NS5B polymerase. Such particles are expected to elicit effective CTL responses against structural and nonstructural HCV protein antigens. This vaccination strategy alone or preferably in conjunction with the subunit strategy described above can be used to elicit high levels of both neutralizing antibodies and CTL responses to help clear the virus. A variety of different HCV genome RNA sequences can be utilized to ensure broadly cross-reactive and protective immune responses. In addition, modification of the HCV particles, either through genetic engineering, or by derivatization *in vitro*, could be used to target infection to cells most effective at eliciting protective and long lasting immune responses.

Live-attenuated HCV derivatives. The ability to manipulate the HCV genome RNA sequence and thereby produce mutants with altered pathogenicity provides a means of constructing live-attenuated HCV variants appropriate for vaccination. Such vaccine candidates express protective antigens but would be impaired in their ability to cause disease, establish chronic infections, trigger autoimmune responses, and transform cells.

Additionally, viruses propagated in cell culture frequently acquire mutations in their RNA genomes that display attenuated phenotypes *in vivo*, while still retaining their immunogenicity. Attenuated virus strains would be impaired in their ability to cause disease and establish chronic infections. Production of HCV variants adapted for tissue culture may represent potential candidates for live-attenuated vaccines. An attractive possibility is the production of HCV derivatives containing the deletion in NS5A described in this application as clone I (see Example 1). Such a variant is less likely to revert to wild type in the host.

HCV Variant-based Gene Expression Vectors

Some of the same properties of HCV leading to chronic liver infection of humans may also be of great utility for designing vectors for gene expression in cell culture systems, genetic vaccination, and gene therapy. The HCV variants described herein can be engineered to produce chimeric RNAs designed for the expression of heterologous gene products (RNAs and proteins). Strategies have been described above and elsewhere [Bredenbeek and Rice, (1992) *supra*; Frolov *et al.*, (1996) *supra*] and include, but are not limited to (i) in-frame fusion of the heterologous coding sequences with the HCV polyprotein; (ii) creation of additional cistrons in the HCV genome RNA; and (iii) inclusion of IRES elements to create multicistronic self-replicating HCV vector RNAs capable of expressing one or more heterologous genes (Figure 2). Functional HCV RNA backbones utilized for such vectors include, but are not limited to, (i) live-attenuated derivatives capable of replication and spread; (ii) RNA replication competent "dead end" derivatives lacking one or more viral components (*e.g.* the structural proteins) required for viral spread; (iii) mutant derivatives capable of high and low levels of HCV-specific RNA synthesis and accumulation; (iv) mutant derivatives adapted for replication in different human cell types; (v) engineered or selected mutant derivatives capable of prolonged noncytopathic replication in human cells. Vectors competent for RNA replication but not packaging or spread can be introduced either as naked RNA, DNA, or packaged into virus-like particles. Such virus-like particles can be produced as described above and composed of either unmodified or altered HCV virion components designed for targeted transfection of the hepatocytes or other human cell types. Alternatively, HCV RNA vectors can be encapsidated and delivered using heterologous viral packaging machineries or encapsulated into liposomes modified for efficient gene delivery. These packaging strategies, and modifications thereof, can be utilized to efficiently target HCV vector RNAs to specific cell types. Using methods detailed above, similar HCV-derived vector systems, competent for replication and expression in other species, can also be derived.

Various methods, *e.g.*, as set forth *supra* in connection with transfection of cells and DNA vaccines, can be used to introduce an HCV vector of the invention. Of primary interest is direct injection of functional HCV RNA or virions, *e.g.*, in the liver. Targeted gene delivery is described in International Patent Publication WO 95/28494, published October 1995. Alternatively, the vector can be introduced *in vivo* by lipofection. For the past decade, there has been increasing use of liposomes for encapsulation and transfection of nucleic acids *in vitro*. Synthetic cationic lipids designed to limit the difficulties and dangers encountered with liposome mediated transfection can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker [Felgner, et. al., *Proc. Natl. Acad. Sci. U.S.A.* 84:7413-7417 (1987); *see* Mackey, *et al., Proc. Natl. Acad. Sci. U.S.A.* 85:8027-8031 (1988); Ulmer *et al., Science* 259:1745-1748 (1993)]. The use of cationic lipids may promote encapsulation of negatively charged nucleic acids, and also promote fusion with negatively charged cell membranes [Felgner and Ringold, *Science* 337:387-388 (1989)]. The use of lipofection to introduce exogenous genes into the specific organs *in vivo* has certain practical advantages. Molecular targeting of liposomes to specific cells represents one area of benefit. It is clear that directing transfection to particular cell types would be particularly advantageous in a tissue with cellular heterogeneity, such as pancreas, liver, kidney, and the brain. Lipids may be chemically coupled to other molecules for the purpose of targeting [*see* Mackey, et. al., *supra*]. Targeted peptides, *e.g.*, hormones or neurotransmitters, and proteins such as antibodies, or non-peptide molecules could be coupled to liposomes chemically. Receptor-mediated DNA delivery approaches can also be used [Curiel *et al., Hum. Gene Ther.* 3:147-154 (1992); Wu and Wu, *J. Biol. Chem.* 262:4429-4432 (1987)].

Examples of applications for gene therapy include, but are not limited to, (i) expression of enzymes or other molecules to correct inherited or acquired metabolic defects; (ii) expression of molecules to promote wound healing; (iii) expression of immunomodulatory molecules to promote immune-mediated regression or elimination of human cancers; (iv) targeted expression of toxic molecules or enzymes capable of activating cytotoxic drugs in tumors; (v) targeted expression of anti-viral or anti-microbial agents in pathogen-infected cells. Various therapeutic heterologous genes can be inserted in a gene therapy vector of the invention, such as but not limited to adenosine deaminase (ADA) to treat severe combined immunodeficiency (SCID); marker genes or lymphokine genes into tumor infiltrating (TIL) T cells [Kasis *et al., Proc. Natl. Acad. Sci. U.S.A.* 87:473 (1990); Culver *et al., ibid.* 88:3155 (1991)]; genes for clotting factors such as Factor VIII and Factor IX for treating hemophilia [Dwarki *et al.Proc. Natl. Acad. Sci. USA*, 92:1023-1027 (19950); Thompson, *Thromb. and Haemostatis*, 66:119-122 (1991)]; and various other well known therapeutic genes such as,

but not limited to, β-globin, dystrophin, insulin, erythropoietin, growth hormone, glucocerebrosidase, β-glucuronidase, α-antitrypsin, phenylalanine hydroxylase, tyrosine hydroxylase, ornithine transcarbamylase, apolipoproteins, and the like. In general, see U.S. Patent No. 5,399,346 to Anderson *et al.*

Examples of applications for genetic vaccination (for protection from pathogens other than HCV) include, but are not limited to, expression of protective antigens from bacterial (*e.g.*, uropathogenic *E. coli, Streptoccoci, Staphlococci, Nisseria*), parasitic (*e.g.*, *Plasmodium, Leishmania, Toxoplama*), fungal (*e.g.*, *Candida, Histoplasma*), and viral (*e.g.*, HIV, HSV, CMV, influenza) human pathogens. Immunogenicity of protective antigens expressed using HCV-derived RNA expression vectors can be enhanced using adjuvants, including co-expression of immunomodulatory molecules, such as cytokines (*e.g.*, IL-2, GM-CSF) to facilitate development of desired Th1 versus Th2 responses. Such adjuvants can be either incorporated and co-expressed by HCV vectors themselves or administered in combination with these vectors using other methods.

Diagnostic Methods for Infectious HCV

Diagnostic cell lines. The invention described herein can also be used to derive cell lines for sensitive diagnosis of infectious HCV in patient samples. In concept, functional HCV components are used to test and create susceptible cell lines (as identified above) in which easily assayed reporter systems are selectively activated upon HCV infection. Examples include, but are not restricted to, (i) defective HCV RNAs lacking replicase components that are incorporated as transgenes and whose replication is upregulated or induced upon HCV infection; and (ii) sensitive heterologous amplifiable reporter systems activated by HCV infection. In the first manifestation, RNA signals required for HCV RNA amplification flank a convenient or a selectable marker (see above). Expression of such chimeric RNAs is driven by an appropriate nuclear promoter and elements required for proper nuclear processing and transport to the cytoplasm. Upon infection of the engineered cell line with HCV, cytoplasmic replication and amplification of the transgene is induced, triggering higher levels of reporter expression, as an indicator of productive HCV infection.

In the second example, cell lines are designed for more tightly regulated but highly inducible reporter gene amplification and expression upon HCV infection. Although this amplfied system is described in the context of specific components, other equivalent components can be used. In one such system, an engineered alphavirus replicon transgene is created which lacks the alphavirus nsP4 polymerase, an enzyme absolutely required for alphavirus RNA amplification and normally produced by cleavage from the nonstructural

polyprotein. Additional features of this defective alphavirus replicon include a subgenomic RNA promoter, driving expression of a luciferase or GFP reporter gene. This promoter element is quiescent in the absence of productive cytoplasmic alphavirus replication. The cell line contains a second transgene for expression of gene fusion consisting of the HCV NS4A protein and the alphavirus nsP4 RDRP. This fused gene is expressed and targeted to the cytoplasmic membrane compartment, but this form of nsP4 would be inactive as a functional component of the alphavirus replication complex because a discrete nsP4 protein, with a precise N terminus is required for nsP4 activity [Lemm *et al., EMBO J.* 13:2925 (1994)]. An optional third transgene expresses a defective alphavirus RNA with *cis* signals for replication, transcription of subgenomic RNA encoding a ubiquitin-nsP4 fusion, and an alphavirus packaging signal. Upon infection of such a cell line by HCV, the HCV NS3 proteinase is produced, mediating *trans* cleavage of the NS4A-nsP4 fusion protein, activating the nsP4 polymerase. This active polymerase, which functions in *trans* and is effective in minute amounts, then forms a functional alphavirus replication complex leading to amplification of the defective alphavirus replicon as well as the defective alphavirus RNA encoding ubiquitin-nsP4. Ubiquitin-nsP4, expressed from its subgenomic RNA, is cleaved efficiently by cellular ubiquitin carboxyterminal hydrolase to product additional nsP4, in case this enzyme is limiting. Once activated, this system would produce extremely high levels of the reporter protein. The time scale of such an HCV infectivity assay is expected to be from hours (for sufficient reporter gene expression).

Antibody diagnostics. In addition to the cell lines described here, HCV variant virus particles (virions) or components thereof, produced by the transfected or infected cell lines, or isolated from an inflected animal, may be used as antigens to detect anti-HCV antibodies in patient blood or blood products. Because the HCV variant virus particles are derived from an authentic HCV genome, particular components such as the coat proteins are likely to have immunogenic properties that more closely resemble or are identical to natural HCV virus than if those components were produced outside of a replicating HCV. Examples of such immunogenic properties include the display of wild-type HCV immunogenic epitopes, and modulation of transcription of genes encoding cellular immune-modulating cytokines. These reagents can be used to establish that a patient is infected with HCV by detecting seroconversion, *i.e.*, generation of a population of HCV-specific antibodies.

Alternatively, antibodies generated to the HCV variant products prepared as described herein can be used to detect the presence of HCV in biological samples from a subject.

Preferred embodiments of the invention are described in the following example. Other embodiments within the scope of the claims herein will be apparent to one skilled in the art from consideration of the specification or practice of the invention as disclosed herein. It is intended that the specification, together with the examples, be considered exemplary only, with the scope and spirit of the invention being indicated by the claims which follow the examples.

Example 1

This example describes the production and evaluation of replicons comprising a *neo* selectable marker and a polyprotein coding region encoding subtype 1b nonstructural proteins.

Materials and Methods

Cell lines. The Huh7 cell lines were generously provided by Robert Lanford (Southwest Foundation for Biomedical Research, San Antonio, U.S.A.) and Ralf Bartenschlager (Johannes Gutenberg University Mainz, Mainz, Germany) and maintained in Dulbecco's modified minimal essential media (DMEM; Gibco-BRL) supplemented with 10% fetal calf serum (FCS), and nonessential amino acids.

Assembly of a selectable subtype 1b replicon. An HCV subtype 1b replicon was constructed which is similar to the replicon described in Lohmann et al., *Science 285*:110-113 (1999). For that construction, a step-wise PCR-based assay utilizing KlenTaqLA DNA polymerase (Wayne Barnes, Washington University) was developed. cDNAs spanning 600-750 bases in length were assembled from 10-12 gel-purified oligonucleotides (60-80 nucleotides in length) with unique complementary overlaps of 16 nucleotides. Four or six oligonucleotides representing the 5' portion of the region to be assembled were annealed and extended in a standard PCR. The remaining six oligonucleotides for the synthesis of the 3' half of the intended cDNA were mixed in a parallel PCR reaction. After 12 cycles of PCR, the extended double-stranded DNA products were combined and subjected to an additional 12 cycles. The product of this reaction resolved as a smear on agarose gels which was excised and the DNA isolated from the agarose. One-fifth of the purified double-stranded DNA product was amplified by PCR using an outer primer pair containing unique restriction enzyme sites to facilitate directional cloning into the pGEM3Zf(+) plasmid vector (Promega). PCR products were purified, digested with appropriate restriction enzymes, and ligated into similarly cleaved pGEM3Zf(+). Multiple recombinant clones were sequenced and the correct clones identified. The overlapping cDNA fragments were assembled into the contiguous replicon sequence. In parallel, a replicon carrying the lethal mutation in the NS5B active site (Gly-Asp-Asp [GDD] to Ala-Ala-Gly [AGG]; pol-) was constructed.

RNA transcription and transfection. RNA transcripts were synthesized in a 100µl reaction mixture containing 40mM Tris-HCl (pH 7.9), 10mM NaCl, 12mM $MgCl_2$, 2mM spermidine, 3mM each ATP, CTP, GTP and UTP, 10mM dithiothreitol, 100 U RNasin (Promega) and 100 U T7 RNA polymerase (Epicentre), and 2µg *Sca I*-linearized DNA. The DNA template was rigorously removed by serial digestions with 30 U DNase I (Boehringer). Ten µg of the DNase-digested RNA transcripts were electroporated into 6x 10^6 Huh7 cells using a model T820 squareporator (BTX), and plated on 150mm dishes. For selection of replicon-containing cells, medium was changed to complete medium containing geneticin (G418; 1mg/ml; Gibco-BRL) at 24 hr post-transfection and thereafter the media was changed every 3-4 days.

RNA analysis. Approximately 5x 10^5 cells were preincubated for 1 h in DMEM lacking phosphate supplemented with 5% dialyzed FCS, 1/20th the normal concentration of phosphate and actinomycin D (4µg/ml; Sigma). [^{32}P]orthophosphate (200µCi/ml; ICN) was added and the incubation continued for an additional 12 h. Total cellular RNA was extracted with TRIZOL, precipitated, and resuspended in H_20 (Gibco-BRL). Radiolabeled RNA was analyzed by denaturing agarose gel electrophoresis and visualized by autoradiography.

Protein analysis. For immunoprecipitation, cell monolayers were incubated for either 4, 8 or 12 h in methionine- and cysteine-deficient MEM containing 1/40th the normal concentration of methionine, 5% dialyzed FCS and Express ^{35}S^{35}S protein labeling mix (100µCi/ml; NEN). Cells were lysed in 100mM $NaPO_4$ pH 7.0 containing 1% sodium dodecyl sulfate (SDS) and protease inhibitors, and cellular DNA sheared by repeated passage through a 27.5 gauge needle. Viral proteins were immunoprecipitated essentially as described previously (Grakoui *etal*, 1993), using patient serum, JHF, recognizing NS3, NS4B and NS5A or rabbit anti-NS5B and Pansorbin cells (Calbiochem). Immunoprecipitates were separated on 10% SDS-PAGE and visualized by autoradiography.

Immunostaining. Cells cultured in 8 well chamber slides (Falcon) were fixed in acetone for 10min at 4°C and allowed to air dry. Rehydrated monolayers were incubated at 37°C with an antibody directed against NS3, followed by incubation with a species-specific fluorescein-conjugated secondary antibody (Pierce), and mounted in 90% glycerol saline containing 50mM Tris-HCl (pH 8.8).

Reverse transcription (RT)-PCR. RNA was isolated from cells using TRIZOL (Gibco-BRL), precipitated and resuspended in H_20. Levels of HCV RNA were quantitated using competitive RT-PCR assays designed to amplify the 5' and 3' NTR sequences of HCV (Kolykhalov *et al*, 1996). For RT-PCR designed to amplify long cDNA fragments, about 1000 molecules of HCV RNA was mixed with the HCV-specific primer, and the primer extended at 43.5°C for 1 h using Superscript II reverse transcriptase (Gibco-BRL). cDNAs were then amplified with KlenTaqLA DNA polymerase using 35 cycles of 95°C for 30 s, 55-

60°C for 30 s, and 68°C for 4 min. PCR products were recovered from preparative low melting-point agarose electrophoresis by phenol extraction, and ~40ng of purified PCR product directly sequenced.

Results

Establishment of G418-resistant colonies. Replicons similar to that described in Lohmann *et al*, *supra*, but derived from the H77 infectious clone, failed to confer resistance to G418 in five different hepatoma cell lines. Sequences of subtype 1b were also used to assemble the replicon I377/NS3-3' (EMBL accession number AJ242652). Replicon RNAs were composed of the HCV internal ribosome entry site (IRES) driving neomycin phosphotransferase gene (Neo) expression and the IRES from encephalomyocarditis virus (EMCV), directing translation of HCV proteins NS3 to NS5B, followed by the 3' NTR) (Figure 3). Two derivatives were constructed which either lacked 2 U nucleotides in the poly (U/UC) tract or carried an *Ava*II restriction enzyme site in the variable region of the 3' NTR, designated HCVrep1bBartMan/Δ2U's and HCVrep1bBartMan/AvaII, respectively. Prior to transfection, translation and correct polyprotein processing was confirmed for each cDNA sequence using the vaccinia-T7 RNA polymerase expression system (data not shown).

DNase-treated replicon RNAs were electroporated into Huh7 cells and after 2-3 weeks in culture G418-resistant colonies were clearly visible. Both replicon derivatives were able to confer G418 resistance, and on average, only 1 in 10^6 cells became G418 resistant. In contrast, colonies were never observed for Huh7 cells electroporated in parallel with the replicon RNAs containing an inactive NS5B polymerase.

Verification of autonomous replication. Twenty two independent colonies were isolated, 5 colonies corresponded to Huh7 cells transfected with RNA transcribed from HCVrep1bBartMan/Δ2U's and the remaining 17 colonies were derived from HCVrep1bBartMan/AvaII RNA. A number of assays were performed to verify that G418 resistance was mediated by autonomously replicating HCV. Amplification of sequences within the 5' and 3' NTRs in a quantitative RT-PCR assay revealed copy numbers ranging

Identification of mutations in HCV replicons. The low frequency of G418-resistant colonies may be attributed to either a cell factor(s) requirement for replication or adaptive changes within the replicon sequence necessary for the establishment of HCV replication. To address the latter possibility, the entire replicon sequence was amplified from cDNA reverse transcribed from RNA isolated from five independent G418-resistant cell clones. Upon direct sequencing of the purified PCR population, multiple mutations were identified. The striking observation was that each cell clone carried a single nucleotide change within NS5A resulting in a coding change (Figure 7). In one instance, a deletion of 47 amino acids (I; Figure 7), encompassing the interferon sensitivity determining region (ISDR), was found. Sequence analysis of NS5A from another 8 G418-resistant cell clones revealed similar point mutations, although 2 clones, which have low levels of HCV replication and slow growth rates (e.g., clone E in Figure 4), were found to contain wild type NS5A. In addition to the identified NS5A mutations, nucleotide substitutions were also noted in NS3 and NS4B; Clone II (SEQ ID NO:9) contains substitutions at nt 3550 (NS3) and nt 4573 (NS4B) (Lys (584) to Glu, and Ser(925) to Gly of SEQ ID NO:3, embodied in SEQ ID NO:17), whereas nt 2060 (NS3) was mutated in Clone VI (Figure 7, corresponding to Gln (87) to Arg of SEQ ID NO:3, embodied in SEQ ID NO:15).

Reconstruction of mutant replicons. To determine if the nucleotide changes and the deletion identified in NS5A were adaptive, each mutation, except mutation II, was independently engineered back into the HCVrep1bBartMan/AvaII backbone. RNA transcribed from each reconstructed replicon was electroporated into naive Huh7 cells, and the number of G418-resistant colonies compared to that obtained for the HCVrep1bBartMan/AvaII replicon containing wild type NS5A. The 47 amino acid deletion, as well as the point mutations, were capable of increasing the frequency of G418-resistant colonies to at least 1% of the initial electroporated cell population (Figure 8), indicating these mutations targeting NS5A are adaptive allowing efficient HCV replication in Huh7 cells. In addition, G418-resistant colonies were observed after transfection of HeLa cells, a human epithelial cell line, with replicon RNA of clone I. Therefore, at least one of the mutations that was adaptive in Huh7 cells also allows the establishment of HCV replication in a non-hepatic cell line.

Example 2

This example describes the production of cell lines permissive for HCV replication; a replicon comprising the NS2 coding region; and full-length HCV cDNA clones comprising the Ser to Ile substitution at position 1179 of SEQ ID NO: 3.

Generation of cell lines. As shown in the previous example, G418-resistant cell clones harboring persistently replicating HCV RNAs were isolated. Two of these G418-resistant cell

clones were treated extensively with the antiviral, interferon-α, to obtain 2 cell lines void of HCV RNA. These are refered to as interferon-treated cell lines I and II.

HCVrep1bBartMan/AvaII, HCV adaptive replicon I or HCV adaptive replicon VII were transfected into the interferon-treated cell lines, I and II. This resulted in a greater G418 transduction efficiency than that observed for the parental Huh-7 cells (see Table 1). Early post-transfection HCV RNA amplification was greatest for the IFN-treated cell line. These results indicate that the cell lines, interferon-treated cell lines I and II, are more permissive for HCV replication than is the parental Huh-7 cell line.

Such cell lines are not only valuable for genetic study of HCV, but also for examining the cellular environments more permissive for HCV replication. For example, microarray technology will allow us to look globally at differences in gene expression profiles between the different cell lines.

Construction of replicons. A replicon was constructed wherein the 5'NTR of HCV was fused to the IRES of EMCV upstream of NS3, thus creating a replicon lacking the neomycin phosphotransferase gene. This replicon, 5'NTR-EMCV/HCVrepVII (SEQ ID NO:25), replicates to high levels in Huh7 cells, as shown in Figure 10. Another replicon, HCVrep/NS2-5B (SEQ ID NO:22) was made wherein the non-structural protein, NS2, is upstream of NS3. As shown in Figure 10, this replicon is also replication-competent in Huh7 cells. This latter replicon can be used advantageously, for example, in testing compounds for inhibiting HCV replication. The addition of the NS2 coding region provides an additional target for such antiviral compounds, as well as providing an additional protein for genetic study.

Full-length HCV RNAs. Two full-length HCV cDNA clones were assembled. The first, HCV FL (SEQ ID NO:24), contains the mutation that encodes a Ser to Ile substitution in NS5A, as shown at position 1179 of SEQ ID NO:3 (see Figure 9). The second, HCV FL-Neo (SEQ ID NO:23), also encodes the Ser to Ile mutation, and in addition, comprises the neomycin phosphotransferase gene immediately 3' of the 5' NTR and the EMCV IRES immediately 5' to the HCV open reading frame (see Figure 9). Both of these full-length clones replicate in the interferon-treated cell line I, as shown in Figure 10. This result indicates that HCV replication is not dependent on the EMCV IRES driving the non-structural proteins of HCV, because the non-structural proteins of the HCV FL clone are driven by the HCV IRES in the full-length clone HCV FL.

In addition, a G418 resistant cell line comprising the HCV FL-Neo clone has been generated from the interferon-treated cell line I described above. This cell line supports high levels of persistently replicating HCV FL-Neo RNA.

All references cited in this specification are hereby incorporated by reference. The discussion of the references herein is intended merely to summarize the assertions made by the authors and no admission is made that any reference constitutes prior art. Applicants reserve the right to challenge the accuracy and pertinence of the cited references.

In view of the above, it will be seen that the several advantages of the invention are achieved and other advantages attained.

As various changes could be made in the above methods and compositions without departing from the scope of the invention, it is intended that all matter contained in the above description and shown in the accompanying drawings and appendix shall be interpreted as illustrative and not in a limiting sense.

Appendix

SEQ ID NOs

SEQ ID NO:1: 5' portion of an HCV 5' NTR

GGCGACACTC CACCATAGAT C

SEQ ID NO:2: 3' portion of a 3' NTR from a wild-type HCV subtype 1a

TGGTGGCTCCATCTTAGCCCTAGTCACGGCTAGCTGTGAAAGGTCCGTGAGCCGC
ATGACTGCAGAGAGTGCTGATACTGGCCTCTCTGCTGATCATGT

SEQ ID NO:3: Amino acid sequence of the polyprotein region of HCVrep1bBartMan

MAPITAYSQQTRGLLGCIITSLTGRDRNQVEGEVQVVSTATQSFLATCVNGVCWTVY
HGAGSKTLAGPKGPITQMYTNVDQDLVGWQAPPGARSLTPCTCGSSDLYLVTRHAD
VIPVRRRGDSRGSLLSPRPVSYLKGSSGGPLLCPSGHAVGIFRAAVCTRGVAKAVDFV
PVESMETTMRSPVFTDNSSPPAVPQTFQVAHLHAPTGSGKSTKVPAAYAAQGYKVL
VLNPSVAATLGFGAYMSKAHGIDPNIRTGVRTITTGAPITYSTYGKFLADGGCSGGAY
DIIICDECHSTDSTTILGIGTVLDQAETAGARLVVLATATPPGSVTVPHPNIEEVALSST
GEIPFYGKAIPIETIKGGRHLIFCHSKKKCDELAAKLSGLGLNAVAYYRGLDVSVIPTS
GDVIVVATDALMTGFTGDFDSVIDCNTCVTQTVDFSLDPTFTIETTTVPQDAVSRSQR
RGRTGRGRMGIYRFVTPGERPSGMFDSSVLCECYDAGCAWYELTPAETSVRLRAYL
NTPGLPVCQDHLEFWESVFTGLTHIDAHFLSQTKQAGDNFPYLVAYQATVCARAQA
PPPSWDQMWKCLIRLKPTLHGPTPLLYRLGAVQNEVTTTHPITKYIMACMSADLEVV
TSTWVLVGGVLAALAAYCLTTGSVVIVGRIILSGKPAIIPDREVLYREFDEMEECASH
LPYIEQGMQLAEQFKQKAIGLLQTATKQAEAAAPVVESKWRTLEAFWAKHMWNFIS
GIQYLAGLSTLPGNPAIASLMAFTASITSPLTTQHTLLFNILGGWVAAQLAPPSAASAF
VGAGIAGAAVGSIGLGKVLVDILAGYGAGVAGALVAFKVMSGEMPSTEDLVNLLPA
ILSPGALVVGVVCAAILRRHVGPGEGAVQWMNRLIAFASRGNHVSPTHYVPESDAA
ARVTQILSSLTITQLLKRLHQWINEDCSTPCSGSWLRDVWDWICTVLTDFKTWLQSK
LLPRLPGVPFFSCQRGYKGVWRGDGIMQTTCPCGAQITGHVKNGSMRIVGPRTCSNT
WHGTFPINAYTTGPCTPSPAPNYSRALWRVAAEEYVEVTRVGDFHYVTGMTTDNVK
CPCQVPAPEFFTEVDGVRLHRYAPACKPLLREEVTFLVGLNQYLVGSQLPCEPEPDV
AVLTSMLTDPSHITAETAKRRLARGSPPSLASSSASQLSAPSLKATCTTRHDSPDADLI
EANLLWRQEMGGNITRVESENKVVILDSFEPLQAEEDEREVSVPAEILRRSRKFPRAM
PIWARPDYNPPLLESWKDPDYVPPVVHGCPLPPAKAPPIPPPRRKRTVVLSESTVSSAL
AELATKTFGSSESSAVDSGTATASPDQPSDDGDAGSDVESYSSMPPLEGEPGDPDLSD
GSWSTVSEEASEDVVCCSMSYTWTGALITPCAAEETKLPINALSNSLLRHHNLVYAT
TSRSASLRQKKVTFDRLQVLDDHYRDVLKEMKAKASTVKAKLLSVEEACKLTPPHS
ARSKFGYGAKDVRNLSSKAVNHIRSVWKDLLEDTETPIDTTIMAKNEVFCVQPEKGG
RKPARLIVFPDLGVRVCEKMALYDVVSTLPQAVMGSSYGFQYSPGQRVEFLVNAWK
AKKCPMGFAYDTRCFDSTVTENDIRVEESIYQCCDLAPEARQAIRSLTERLYIGGPLT
NSKGQNCGYRRCRASGVLTTSCGNTLTCYLKAAAACRAAKLQDCTMLVCGDDLVV
ICESAGTQEDEASLRAFTEAMTRYSAPPGDPPKPEYDLELITSCSSNVSVAHDASGKR
VYYLTRDPTTPLARAAWETARHTPVNSWLGNIIMYAPTLWARMILMTHFFSILLAQE
QLEKALDCQIYGACYSIEPLDLPQIIQRLHGLSAFSLHSYSPGEINRVASCLRKLGVPPL
RVWRHRARSVRARLLSQGGRAATCGKYLFNWAVRTKLKLTPIPAASQLDLSSWFVA
GYSGGDIYHSLSRARPRWFMWCLLLLSVGVGIYLLPNR

SEQ ID NO:4: Amino acid sequence of the NS5A protein of HCVrep1bBartMan

SGSWLRDVWDWICTVLTDFKTWLQSKLLPRLPGVPFFSCQRGYKGVWRGDGIMQTT
CPCGAQITGHVKNGSMRIVGPRTCSNTWHGTFPINAYTTGPCTPSPAPNYSRALWRV
AAEEYVEVTRVGDFHYVTGMTTDNVKCPCQVPAPEFFTEVDGVRLHRYAPACKPLL
REEVTFLVGLNQYLVGSQLPCEPEPDVAVLTSMLTDPSHITAETAKRRLARGSPPSLA
SSSASQLSAPSLKATCTTRHDSPDADLIEANLLWRQEMGGNITRVESENKVVILDSFE
PLQAEEDEREVSVPAEILRRSRKFPRAMPIWARPDYNPPLLESWKDPDYVPPVVHGCP
LPPAKAPPIPPPRRKRTVVLSESTVSSALAELATKTFGSSESSAVDSGTATASPDQPSD
DGDAGSDVESYSSMPPLEGEPGDPDLSDGSWSTVSEEASEDVVCC

SEQ ID NO:5: Nucleotide sequence of DNA clone of HCVrep1bBartMan/Δ2U's

GCCAGCCCCCGATTGGGGGCGACACTCCACCATAGATCACTCCCCTGTGAGGAAC
TACTGTCTTCACGCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTGCAG
CCTCCAGGACCCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTA
CACCGGAATTGCCAGGACGACCGGGTCCTTTCTTGGATCAACCCGCTCAATGCCT
GGAGATTTGGGCGTGCCCCCGCGAGACTGCTAGCCGAGTAGTGTTGGGTCGCGA
AAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCCCCGGGAGGTCTCGT
AGACCGTGCACCATGAGCACGAATCCTAAACCTCAAAGAAAAACCAAAGGGCGC
GCCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGA
GGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGT
GTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCC
GGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACG
ACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACT
GGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCC
TGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGAT
CCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGT
ACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAG
GGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGC
GAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAA
ATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTA
TCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGG
GCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGC
CTTCTATCGCCTTCTTGACGAGTTCTTCTGAGTTTAAACAGACCACAACGGTTTCC
CTCTAGCGGGATCAATTCCGCCCCTCTCCCTCCCCCCCCCCTAACGTTACTGGCCG
AAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATAT
TGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAG
CATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTC
GTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCG
ACCCTTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAA
AGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTG
TGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAA
GGGGCTGAAGGATGCCCAGAAGGTACCCCATTGTATGGGATCTGATCTGGGGCCT
CGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCCCC
GAACCACGGGGACGTGGTTTTCCTTTGAAAAACACGATAATACCATGGCGCCTAT
TACGGCCTACTCCCAACAGACGCGAGGCCTACTTGGCTGCATCATCACTAGCCTC
ACAGGCCGGGACAGGAACCAGGTCGAGGGGGAGGTCCAAGTGGTCTCCACCGCA
ACACAATCTTTCCTGGCGACCTGCGTCAATGGCGTGTGTTGGACTGTCTATCATG
GTGCCGGCTCAAAGACCCTTGCCGGCCCAAAGGGCCCAATCACCCAAATGTACA

CCAATGTGGACCAGGACCTCGTCGGCTGGCAAGCGCCCCCCGGGGCGCGTTCCTT
GACACCATGCACCTGCGGCAGCTCGGACCTTTACTTGGTCACGAGGCATGCCGAT
GTCATTCCGGTGCGCCGGCGGGGCGACAGCAGGGGGAGCCTACTCTCCCCCAGG
CCCGTCTCCTACTTGAAGGGCTCTTCGGGCGGTCCACTGCTCTGCCCCTCGGGGC
ACGCTGTGGGCATCTTTCGGGCTGCCGTGTGCACCCGAGGGGTTGCGAAGGCGGT
GGACTTTGTACCCGTCGAGTCTATGGAAACCACTATGCGGTCCCCGGTCTTCACG
GACAACTCGTCCCCTCCGGCCGTACCGCAGACATTCCAGGTGGCCCATCTACACG
CCCCTACTGGTAGCGGCAAGAGCACTAAGGTGCCGGCTGCGTATGCAGCCCAAG
GGTATAAGGTGCTTGTCCTGAACCCGTCCGTCGCCGCCACCCTAGGTTTCGGGGC
GTATATGTCTAAGGCACATGGTATCGACCCTAACATCAGAACCGGGGTAAGGAC
CATCACCACGGGTGCCCCCATCACGTACTCCACCTATGGCAAGTTTCTTGCCGAC
GGTGGTTGCTCTGGGGGCGCCTATGACATCATAATATGTGATGAGTGCCACTCAA
CTGACTCGACCACTATCCTGGGCATCGGCACAGTCCTGGACCAAGCGGAGACGG
CTGGAGCGCGACTCGTCGTGCTCGCCACCGCTACGCCTCCGGGATCGGTCACCGT
GCCACATCCAAACATCGAGGAGGTGGCTCTGTCCAGCACTGGAGAAATCCCCTTT
TATGGCAAAGCCATCCCCATCGAGACCATCAAGGGGGGGAGGCACCTCATTTTCT
GCCATTCCAAGAAGAAATGTGATGAGCTCGCCGCGAAGCTGTCCGGCCTCGGACT
CAATGCTGTAGCATATTACCGGGGCCTTGATGTATCCGTCATACCAACTAGCGGA
GACGTCATTGTCGTAGCAACGGACGCTCTAATGACGGGCTTTACCGGCGATTTCG
ACTCAGTGATCGACTGCAATACATGTGTCACCCAGACAGTCGACTTCAGCCTGGA
CCCGACCTTCACCATTGAGACGACGACCGTGCCACAAGACGCGGTGTCACGCTCG
CAGCGGCGAGGCAGGACTGGTAGGGGCAGGATGGGCATTTACAGGTTTGTGACT
CCAGGAGAACGGCCCTCGGGCATGTTCGATTCCTCGGTTCTGTGCGAGTGCTATG
ACGCGGGCTGTGCTTGGTACGAGCTCACGCCCGCCGAGACCTCAGTTAGGTTGCG
GGCTTACCTAAACACACCAGGGTTGCCCGTCTGCCAGGACCATCTGGAGTTCTGG
GAGAGCGTCTTTACAGGCCTCACCCACATAGACGCCCATTTCTTGTCCCAGACTA
AGCAGGCAGGAGACAACTTCCCCTACCTGGTAGCATACCAGGCTACGGTGTGCG
CCAGGGCTCAGGCTCCACCTCCATCGTGGGACCAAATGTGGAAGTGTCTCATACG
GCTAAAGCCTACGCTGCACGGGCCAACGCCCCTGCTGTATAGGCTGGGAGCCGTT
CAAAACGAGGTTACTACCACACACCCCATAACCAAATACATCATGGCATGCATGT
CGGCTGACCTGGAGGTCGTCACGAGCACCTGGGTGCTGGTAGGCGGAGTCCTAG
CAGCTCTGGCCGCGTATTGCCTGACAACAGGCAGCGTGGTCATTGTGGGCAGGAT
CATCTTGTCCGGAAAGCCGGCCATCATTCCCGACAGGGAAGTCCTTTACCGGGAG
TTCGATGAGATGGAAGAGTGCGCCTCACACCTCCCTTACATCGAACAGGGAATGC
AGCTCGCCGAACAATTCAAACAGAAGGCAATCGGGTTGCTGCAAACAGCCACCA
AGCAAGCGGAGGCTGCTGCTCCCGTGGTGGAATCCAAGTGGCGGACCCTCGAAG
CCTTCTGGGCGAAGCATATGTGGAATTTCATCAGCGGGATACAATATTTAGCAGG
CTTGTCCACTCTGCCTGGCAACCCCGCGATAGCATCACTGATGGCATTCACAGCC
TCTATCACCAGCCCGCTCACCACCCAACATACCCTCCTGTTTAACATCCTGGGGG
GATGGGTGGCCGCCCAACTTGCTCCTCCCAGCGCTGCTTCTGCTTTCGTAGGCGCC
GGCATCGCTGGAGCGGCTGTTGGCAGCATAGGCCTTGGGAAGGTGCTTGTGGATA
TTTTGGCAGGTTATGGAGCAGGGGTGGCAGGCGCGCTCGTGGCCTTTAAGGTCAT
GAGCGGCGAGATGCCCTCCACCGAGGACCTGGTTAACCTACTCCCTGCTATCCTC
TCCCCTGGCGCCCTAGTCGTCGGGGTCGTGTGCGCAGCGATACTGCGTCGGCACG
TGGGCCCAGGGGAGGGGGCTGTGCAGTGGATGAACCGGCTGATAGCGTTCGCTT
CGCGGGGTAACCACGTCTCCCCCACGCACTATGTGCCTGAGAGCGACGCTGCAGC
ACGTGTCACTCAGATCCTCTCTAGTCTTACCATCACTCAGCTGCTGAAGAGGCTTC
ACCAGTGGATCAACGAGGACTGCTCCACGCCATGCTCCGGCTCGTGGCTAAGAG
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CAAGCTCCTGCCGCGATTGCCGGGAGTCCCCTTCTTCTCATGTCAACGTGGGTAC
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TTTTTCTCCTTTTTTTTTCCTCTTTTTTTCCTTTTCTTTCCTTTGGTGGCTCCATCTTA
GCCCTAGTCACGGCTAGCTGTGAAAGGTCCGTGAGCCGCTTGACTGCAGAGAGTG
CTGATACTGGCCTCTCTGCAGATCAAGT

SEQ ID NO:6: Nucleotide sequence of DNA clone of HCVrep1bBartMan/AvaII, where the nucleotide change creating the AvaII site is in lower case and highlighted in bold

GCCAGCCCCCGATTGGGGGCGACACTCCACCATAGATCACTCCCCTGTGAGGAAC
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<u>SEQ ID NO:7</u>: Nucleotide sequence of DNA clone of HCV adaptive replicon I, where the amino acid generated by the deletion is identified in lower case and highlighted in bold

GCCAGCCCCCGATTGGGGGCGACACTCCACCATAGATCACTCCCCTGTGAGGAAC
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<u>SEQ ID NO:8</u>: Nucleotide sequence of DNA clone of HCV adaptive replicon VI, where nucleotide changes are in lower case and highlighted in bold

GCCAGCCCCCGATTGGGGGCGACACTCCACCATAGATCACTCCCCTGTGAGGAAC
TACTGTCTTCACGCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTGCAG
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SEQ ID NO:9: Nucleotide sequence of DNA clone of HCV adaptive replicon II, where nucleotide changes are in lower case and highlighted in bold

GCCAGCCCCCGATTGGGGGCGACACTCCACCATAGATCACTCCCCTGTGAGGAAC
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TTGGACTCTTTCGAGCCGCTCCAAGCGGAGGAGGATGAGAGGGAAGTATCCGTTC
CGGCGGAGATCCTGCGGAGGTCCAGGAAATTCCCTCGAGCGATGCCCATATGGG
CACGCCCGGATTACAACCCTCCACTGTTAGAGTCCTGGAAGGACCCGGACTACGT
CCCTCCAGTGGTACACGGGTGTCCATTGCCGCCTGCCAAGGCCCCTCCGATACCA
CCTCCACGGAGGAAGAGGACGGTTGTCCTGTCAGAATCTACCGTGTCTTCTGCCT
TGGCGGAGCTCGCCACAAAGACCTTCGGCAGCTCCGAATCGTCGGCCGTCGACA
GCGGCACGGCAACGGCCTCTCCTGACCAGCCCTCCGACGACGGCGACGCGGGAT
CCGACGTTGAGTCGTACTCCTCCATGCCCCCCCTTGAGGGGGAGCCGGGGGATCC
CGATCTCAGCGACGGGTCTTGGTCTACCGTAAGCGAGGAGGCTAGTGAGGACGT
CGTCTGCTGCTCGATGTCCTACACATGGACAGGCGCCCTGATCACGCCATGCGCT
GCGGAGGAAACCAAGCTGCCCATCAATGCACTGAGCAACTCTTTGCTCCGTCACC
ACAACTTGGTCTATGCTACAACATCTCGCAGCGCAAGCCTGCGGCAGAAGAAGG
TCACCTTTGACAGACTGCAGGTCCTGGACGACCACTACCGGGACGTGCTCAAGGA
GATGAAGGCGAAGGCGTCCACAGTTAAGGCTAAACTTCTATCCGTGGAGGAAGC
CTGTAAGCTGACGCCCCCACATTCGGCCAGATCTAAATTTGGCTATGGGGCAAAG
GACGTCCGGAACCTATCCAGCAAGGCCGTTAACCACATCCGCTCCGTGTGGAAGG
ACTTGCTGGAAGACACTGAGACACCAATTGACACCACCATCATGGCAAAAAATG
AGGTTTTCTGCGTCCAACCAGAGAAGGGGGGCCGCAAGCCAGCTCGCCTTATCGT
ATTCCCAGATTTGGGGGTTCGTGTGTGCGAGAAAATGGCCCTTTACGATGTGGTC
TCCACCCTCCCTCAGGCCGTGATGGGCTCTTCATACGGATTCCAATACTCTCCTGG
ACAGCGGGTCGAGTTCCTGGTGAATGCCTGGAAAGCGAAGAAATGCCCTATGGG
CTTCGCATATGACACCCGCTGTTTTGACTCAACGGTCACTGAGAATGACATCCGT
GTTGAGGAGTCAATCTACCAATGTTGTGACTTGGCCCCCGAAGCCAGACAGGCCA
TAAGGTCGCTCACAGAGCGGCTTTACATCGGGGGCCCCCTGACTAATTCTAAAGG
GCAGAACTGCGGCTATCGCCGGTGCCGCGAGCGGTGTACTGACGACCAGCTG

CGGTAATACCCTCACATGTTACTTGAAGGCCGCTGCGGCCTGTCGAGCTGCGAAG
CTCCAGGACTGCACGATGCTCGTATGCGGAGACGACCTTGTCGTTATCTGTGAAA
GCGCGGGGACCCAAGAGGACGAGGCGAGCCTACGGGCCTTCACGGAGGCTATGA
CTAGATACTCTGCCCCCCCTGGGGACCCGCCCAAACCAGAATACGACTTGGAGTT
GATAACATCATGCTCCTCCAATGTGTCAGTCGCGCACGATGCATCTGGCAAAAGG
GTGTACTATCTCACCCGTGACCCCACCACCCCCCTTGCGCGGGCTGCGTGGAGA
CAGCTAGACACACTCCAGTCAATTCCTGGCTAGGCAACATCATCATGTATGCGCC
CACCTTGTGGGCAAGGATGATCCTGATGACTCATTTCTTCTCCATCCTTCTAGCTC
AGGAACAACTTGAAAAAGCCCTAGATTGTCAGATCTACGGGGCCTGTTACTCCAT
TGAGCCACTTGACCTACCTCAGATCATTCAACGACTCCATGGCCTTAGCGCATTTT
CACTCCATAGTTACTCTCCAGGTGAGATCAATAGGGTGGCTTCATGCCTCAGGAA
ACTTGGGGTACCGCCCTTGCGAGTCTGGAGACATCGGGCCAGAAGTGTCCGCGCT
AGGCTACTGTCCCAGGGGGGGAGGGCTGCCACTTGTGGCAAGTACCTCTTCAACT
GGGCAGTAAGGACCAAGCTCAAACTCACTCCAATCCCGGCTGCGTCCCAGTTGGA
TTTATCCAGCTGGTTCGTTGCTGGTTACAGCGGGGGAGACATATATCACAGCCTG
TCTCGTGCCCGACCCCGCTGGTTCATGTGGTGCCTACTCCTACTTTCTGTAGGGGT
AGGCATCTATCTACTCCCCAACCGATGAACGGGGACCTAAACACTCCAGGCCAAT
AGGCCATCCTGTTTTTTTCCCTTTTTTTTTTTCTTTTTTTTTTTTTTTTTTTTTTTTT
TTTTTTTCTCCTTTTTTTTTCCTCTTTTTTTCCTTTTCTTTCCTTTGGTGGCTCCATCT
TAGCCCTAGTCACGGCTAGCTGTGAAAGGTCCGTGAGCCGCTTGACTGCAGAGAG
TGCTGATACTGGCCTCTCTGCAGATCAAGT

SEQ ID NO:10: Nucleotide sequence of DNA clone of HCV adaptive replicon V, where nucleotide change is in lower case and highlighted in bold

GCCAGCCCCCGATTGGGGGCGACACTCCACCATAGATCACTCCCCTGTGAGGAAC
TACTGTCTTCACGCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTGCAG
CCTCCAGGACCCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTA
CACCGGAATTGCCAGGACGACCGGGTCCTTTCTTGGATCAACCCGCTCAATGCCT
GGAGATTTGGGCGTGCCCCCGCGAGACTGCTAGCCGAGTAGTGTTGGGTCGCGA
AAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCCCCGGGAGGTCTCGT
AGACCGTGCACCATGAGCACGAATCCTAAACCTCAAAGAAAAACCAAAGGGCGC
GCCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGA
GGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGT
GTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCC
GGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACG
ACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACT
GGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCC
TGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGAT
CCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGT
ACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAG
GGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGC
GAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAA
ATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTA
TCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGG
GCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGC
CTTCTATCGCCTTCTTGACGAGTTCTTCTGAGTTTAAACAGACCACAACGGTTTCC
CTCTAGCGGGATCAATTCCGCCCCTCTCCCTCCCCCCCCCCTAACGTTACTGGCCG
AAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATAT
TGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAG
CATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTC
GTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCG

ACCCTTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAA
AGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTG
TGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAA
GGGGCTGAAGGATGCCCAGAAGGTACCCCATTGTATGGGATCTGATCTGGGGCCT
CGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCCCC
GAACCACGGGGACGTGGTTTTCCTTTGAAAAACACGATAATACCATGGCGCCTAT
TACGGCCTACTCCCAACAGACGCGAGGCCTACTTGGCTGCATCATCACTAGCCTC
ACAGGCCGGGACAGGAACCAGGTCGAGGGGGAGGTCCAAGTGGTCTCCACCGCA
ACACAATCTTTCCTGGCGACCTGCGTCAATGGCGTGTGTTGGACTGTCTATCATG
GTGCCGGCTCAAAGACCCTTGCCGGCCCAAAGGGCCCAATCACCCAAATGTACA
CCAATGTGGACCAGGACCTCGTCGGCTGGCAAGCGCCCCCCGGGGCGCGTTCCTT
GACACCATGCACCTGCGGCAGCTCGGACCTTTACTTGGTCACGAGGCATGCCGAT
GTCATTCCGGTGCGCCGGCGGGGCGACAGCAGGGGGAGCCTACTCTCCCCCAGG
CCCGTCTCCTACTTGAAGGGCTCTTCGGGCGGTCCACTGCTCTGCCCCTCGGGGC
ACGCTGTGGGCATCTTTCGGGCTGCCGTGTGCACCCGAGGGGTTGCGAAGGCGGT
GGACTTTGTACCCGTCGAGTCTATGGAAACCACTATGCGGTCCCCGGTCTTCACG
GACAACTCGTCCCCTCCGGCCGTACCGCAGACATTCCAGGTGGCCCATCTACACG
CCCCTACTGGTAGCGGCAAGAGCACTAAGGTGCCGGCTGCGTATGCAGCCCAAG
GGTATAAGGTGCTTGTCCTGAACCCGTCCGTCGCCGCCACCCTAGGTTTCGGGGC
GTATATGTCTAAGGCACATGGTATCGACCCTAACATCAGAACCGGGGTAAGGAC
CATCACCACGGGTGCCCCCATCACGTACTCCACCTATGGCAAGTTTCTTGCCGAC
GGTGGTTGCTCTGGGGGCGCCTATGACATCATAATATGTGATGAGTGCCACTCAA
CTGACTCGACCACTATCCTGGGCATCGGCACAGTCCTGGACCAAGCGGAGACGG
CTGGAGCGCGACTCGTCGTGCTCGCCACCGCTACGCCTCCGGGATCGGTCACCGT
GCCACATCCAAACATCGAGGAGGTGGCTCTGTCCAGCACTGGAGAAATCCCCTTT
TATGGCAAAGCCATCCCCATCGAGACCATCAAGGGGGGGAGGCACCTCATTTTCT
GCCATTCCAAGAAGAAATGTGATGAGCTCGCCGCGAAGCTGTCCGGCCTCGGACT
CAATGCTGTAGCATATTACCGGGGCCTTGATGTATCCGTCATACCAACTAGCGGA
GACGTCATTGTCGTAGCAACGGACGCTCTAATGACGGGCTTTACCGGCGATTTCG
ACTCAGTGATCGACTGCAATACATGTGTCACCCAGACAGTCGACTTCAGCCTGGA
CCCGACCTTCACCATTGAGACGACGACCGTGCCACAAGACGCGGTGTCACGCTCG
CAGCGGCGAGGCAGGACTGGTAGGGGCAGGATGGGCATTTACAGGTTTGTGACT
CCAGGAGAACGGCCCTCGGGCATGTTCGATTCCTCGGTTCTGTGCGAGTGCTATG
ACGCGGGCTGTGCTTGGTACGAGCTCACGCCCGCCGAGACCTCAGTTAGGTTGCG
GGCTTACCTAAACACACCAGGGTTGCCCGTCTGCCAGGACCATCTGGAGTTCTGG
GAGAGCGTCTTTACAGGCCTCACCCACATAGACGCCCATTTCTTGTCCCAGACTA
AGCAGGCAGGAGACAACTTCCCCTACCTGGTAGCATACCAGGCTACGGTGTGCG
CCAGGGCTCAGGCTCCACCTCCATCGTGGGACCAAATGTGGAAGTGTCTCATACG
GCTAAAGCCTACGCTGCACGGGCCAACGCCCCTGCTGTATAGGCTGGGAGCCGTT
CAAAACGAGGTTACTACCACACACCCCATAACCAAATACATCATGGCATGCATGT
CGGCTGACCTGGAGGTCGTCACGAGCACCTGGGTGCTGGTAGGCGGAGTCCTAG
CAGCTCTGGCCGCGTATTGCCTGACAACAGGCAGCGTGGTCATTGTGGGCAGGAT
CATCTTGTCCGGAAAGCCGGCCATCATTCCCGACAGGGAAGTCCTTTACCGGGAG
TTCGATGAGATGGAAGAGTGCGCCTCACACCTCCCTTACATCGAACAGGGAATGC
AGCTCGCCGAACAATTCAAACAGAAGGCAATCGGGTTGCTGCAAACAGCCACCA
AGCAAGCGGAGGCTGCTGCTCCCGTGGTGGAATCCAAGTGGCGGACCCTCGAAG
CCTTCTGGGCGAAGCATATGTGGAATTTCATCAGCGGGATACAATATTTAGCAGG
CTTGTCCACTCTGCCTGGCAACCCCGCGATAGCATCACTGATGGCATTCACAGCC
TCTATCACCAGCCCGCTCACCACCCAACATACCCTCCTGTTTAACATCCTGGGGG
GATGGGTGGCCGCCCAACTTGCTCCTCCCAGCGCTGCTTCTGCTTTCGTAGGCGCC
GGCATCGCTGGAGCGGCTGTTGGCAGCATAGGCCTTGGGAAGGTGCTTGTGGATA
TTTTGGCAGGTTATGGAGCAGGGGTGGCAGGCGCGCTCGTGGCCTTTAAGGTCAT
GAGCGGCGAGATGCCCTCCACCGAGGACCTGGTTAACCTACTCCCTGCTATCCTC

TCCCCTGGCGCCCTAGTCGTCGGGGTCGTGTGCGCAGCGATACTGCGTCGGCACG
TGGGCCCAGGGGAGGGGGCTGTGCAGTGGATGAACCGGCTGATAGCGTTCGCTT
CGCGGGGTAACCACGTCTCCCCCACGCACTATGTGCCTGAGAGCGACGCTGCAGC
ACGTGTCACTCAGATCCTCTCTAGTCTTACCATCACTCAGCTGCTGAAGAGGCTTC
ACCAGTGGATCAACGAGGACTGCTCCACGCCATGCTCCGGCTCGTGGCTAAGAG
ATGTTTGGGATTGGATATGCACGGTGTTGACTGATTTCAAGACCTGGCTCCAGTC
CAAGCTCCTGCCGCGATTGCCGGGAGTCCCCTTCTTCTCATGTCAACGTGGGTAC
AAGGGAGTCTGGCGGGGCGACGGCATCATGCAAACCACCTGCCCATGTGGAGCA
CAGATCACCGGACATGTGAAAAACGGTTCCATGAGGATCGTGGGGCCTAGGACC
TGTAGTAACACGTGGCATGGAACATTCCCCATTAACGCGTACACCACGGGCCCCT
GCACGCCCTCCCCGGCGCCAAATTATTCTAGGGCGCTGTGGCGGGTGGCTGCTGA
GGAGTACGTGGAGGTTACGCGGGTGGGGGATTTCCACTACGTGACGGGCATGAC
CACTGACAACGTAAAGTGCCCGTGTCAGGTTCCGGCCCCCGAATTCTTCACAGAA
GTGGATGGGGTGCGGTTGCACAGGTACGCTCCAGCGTGCAAACCCCTCCTACGGG
AGGAGGTCACATTCCTGGTCGGGCTCAATCAATACCTGGTTGGGTCACAGCTCCC
ATGCGAGCCCGAACCGGACGTAGCAGTGCTCACTTCCATGCTCACCGACCCCTCC
CACATTACGGCGGAGACGGCTAAGCGTAGGCTGGCCAGGGGATCTCCCCCCTCCT
TGtCCAGCTCATCAGCTAGCCAGCTGTCTGCGCCTTCCTTGAAGGCAACATGCACT
ACCCGTCATGACTCCCCGGACGCTGACCTCATCGAGGCCAACCTCCTGTGGCGGC
AGGAGATGGGCGGGAACATCACCCGCGTGGAGTCAGAAAATAAGGTAGTAATTT
TGGACTCTTTCGAGCCGCTCCAAGCGGAGGAGGATGAGAGGGAAGTATCCGTTC
CGGCGGAGATCCTGCGGAGGTCCAGGAAATTCCCTCGAGCGATGCCCATATGGG
CACGCCCGGATTACAACCCTCCACTGTTAGAGTCCTGGAAGGACCCGGACTACGT
CCCTCCAGTGGTACACGGGTGTCCATTGCCGCCTGCCAAGGCCCCTCCGATACCA
CCTCCACGGAGGAAGAGGACGGTTGTCCTGTCAGAATCTACCGTGTCTTCTGCCT
TGGCGGAGCTCGCCACAAAGACCTTCGGCAGCTCCGAATCGTCGGCCGTCGACA
GCGGCACGGCAACGGCCTCTCCTGACCAGCCCTCCGACGACGGCGACGCGGGAT
CCGACGTTGAGTCGTACTCCTCCATGCCCCCCCTTGAGGGGGAGCCGGGGGATCC
CGATCTCAGCGACGGGTCTTGGTCTACCGTAAGCGAGGAGGCTAGTGAGGACGT
CGTCTGCTGCTCGATGTCCTACACATGGACAGGCGCCCTGATCACGCCATGCGCT
GCGGAGGAAACCAAGCTGCCCATCAATGCACTGAGCAACTCTTTGCTCCGTCACC
ACAACTTGGTCTATGCTACAACATCTCGCAGCGCAAGCCTGCGGCAGAAGAAGG
TCACCTTTGACAGACTGCAGGTCCTGGACGACCACTACCGGGACGTGCTCAAGGA
GATGAAGGCGAAGGCGTCCACAGTTAAGGCTAAACTTCTATCCGTGGAGGAAGC
CTGTAAGCTGACGCCCCCACATTCGGCCAGATCTAAATTTGGCTATGGGGCAAAG
GACGTCCGGAACCTATCCAGCAAGGCCGTTAACCACATCCGCTCCGTGTGGAAGG
ACTTGCTGGAAGACACTGAGACACCAATTGACACCACCATCATGGCAAAAAATG
AGGTTTTCTGCGTCCAACCAGAGAAGGGGGGCCGCAAGCCAGCTCGCCTTATCGT
ATTCCCAGATTTGGGGGTTCGTGTGTGCGAGAAAATGGCCCTTTACGATGTGGTC
TCCACCCTCCCTCAGGCCGTGATGGGCTCTTCATACGGATTCCAATACTCTCCTGG
ACAGCGGGTCGAGTTCCTGGTGAATGCCTGGAAAGCGAAGAAATGCCCTATGGG
CTTCGCATATGACACCCGCTGTTTTGACTCAACGGTCACTGAGAATGACATCCGT
GTTGAGGAGTCAATCTACCAATGTTGTGACTTGGCCCCCGAAGCCAGACAGGCCA
TAAGGTCGCTCACAGAGCGGCTTTACATCGGGGGCCCCCTGACTAATTCTAAAGG
GCAGAACTGCGGCTATCGCCGGTGCCGCGCGAGCGGTGTACTGACGACCAGCTG
CGGTAATACCCTCACATGTTACTTGAAGGCCGCTGCGGCCTGTCGAGCTGCGAAG
CTCCAGGACTGCACGATGCTCGTATGCGGAGACGACCTTGTCGTTATCTGTGAAA
GCGCGGGGACCCAAGAGGACGAGGCGAGCCTACGGGCCTTCACGGAGGCTATGA
CTAGATACTCTGCCCCCCCTGGGGACCCGCCCAAACCAGAATACGACTTGGAGTT
GATAACATCATGCTCCTCCAATGTGTCAGTCGCGCACGATGCATCTGGCAAAAGG
GTGTACTATCTCACCCGTGACCCCACCACCCCCCTTGCGCGGGCTGCGTGGGAGA
CAGCTAGACACACTCCAGTCAATTCCTGGCTAGGCAACATCATCATGTATGCGCC
CACCTTGTGGGCAAGGATGATCCTGATGACTCATTTCTTCTCCATCCTTCTAGCTC

AGGAACAACTTGAAAAAGCCCTAGATTGTCAGATCTACGGGGCCTGTTACTCCAT
TGAGCCACTTGACCTACCTCAGATCATTCAACGACTCCATGGCCTTAGCGCATTTT
CACTCCATAGTTACTCTCCAGGTGAGATCAATAGGGTGGCTTCATGCCTCAGGAA
ACTTGGGGTACCGCCCTTGCGAGTCTGGAGACATCGGGCCAGAAGTGTCCGCGCT
AGGCTACTGTCCCAGGGGGGGAGGGCTGCCACTTGTGGCAAGTACCTCTTCAACT
GGGCAGTAAGGACCAAGCTCAAACTCACTCCAATCCCGGCTGCGTCCCAGTTGGA
TTTATCCAGCTGGTTCGTTGCTGGTTACAGCGGGGGAGACATATATCACAGCCTG
TCTCGTGCCCGACCCCGCTGGTTCATGTGGTGCCTACTCCTACTTTCTGTAGGGGT
AGGCATCTATCTACTCCCCAACCGATGAACGGGGACCTAAACACTCCAGGCCAAT
AGGCCATCCTGTTTTTTTCCCTTTTTTTTTTTCTTTTTTTTTTTTTTTTTTTTTTTTT
TTTTTTTCTCCTTTTTTTTTCCTCTTTTTTTCCTTTTCTTTCCTTTGGTGGCTCCATCT
TAGCCCTAGTCACGGCTAGCTGTGAAAGGTCCGTGAGCCGCTTGACTGCAGAGAG
TGCTGATACTGGCCTCTCTGCAGATCAAGT

SEQ ID NO:11: NS5A gene of DNA clone of HCV adaptive replicon IV, where nucleotide change is in lower case and highlighted in bold

TCCGGCTCGTGGCTAAGAGATGTTTGGGATTGGATATGCACGGTGTTGACTGATT
TCAAGACCTGGCTCCAGTCCAAGCTCCTGCCGCGATTGCCGGGAGTCCCCTTCTT
CTCATGTCAACGTGGGTACAAGGGAGTCTGGCGGGGCGACGGCATCATGCAAAC
CACCTGCCCATGTGGAGCACAGATCACCGGACATGTGAAAAACGGTTCCATGAG
GATCGTGGGGCCTAGGACCTGTAGTAACACGTGGCATGGAACATTCCCCATTAAC
GCGTACACCACGGGCCCCTGCACGCCCTCCCCGGCGCCAAATTATTCTAGGGCGC
TGTGGCGGGTGGCTGCTGAGGAGTACGTGGAGGTTACGCGGGTGGGGGATTTCC
ACTACGTGACGGGCATGACCACTGACAACGTAAAGTGCCCGTGTCAGGTTCCGGC
CCCCGAATTCTTCACAGAAGTGGATGGGGTGCGGTTGCACAGGTACGCTCCAGCG
TGCAAACCCCTCCTACGGGAGGAGGTCACATTCCTGGTCGGGCTCAATCAATACC
TGGTTGGGTCACAGCTCCCATGCGAGCCCGAACCGGACGTAGCAGTGCTCACTTC
CATGCTCACCGACCCCTCCCACATTACGGCGGAGACGGCTAAGCGTAGGCTGGCC
AGGGGATCTCCCCCCCT**g**CTTGGCCAGCTCATCAGCTAGCCAGCTGTCTGCGCCTTC
CTTGAAGGCAACATGCACTACCCGTCATGACTCCCCGGACGCTGACCTCATCGAG
GCCAACCTCCTGTGGCGGCAGGAGATGGGCGGGAACATCACCCGCGTGGAGTCA
GAAAATAAGGTAGTAATTTTGGACTCTTTCGAGCCGCTCCAAGCGGAGGAGGAT
GAGAGGGAAGTATCCGTTCCGGCGGAGATCCTGCGGAGGTCCAGGAAATTCCCT
CGAGCGATGCCCATATGGGCACGCCCGGATTACAACCCTCCACTGTTAGAGTCCT
GGAAGGACCCGGACTACGTCCCTCCAGTGGTACACGGGTGTCCATTGCCGCCTGC
CAAGGCCCCTCCGATACCACCTCCACGGAGGAAGAGGACGGTTGTCCTGTCAGA
ATCTACCGTGTCTTCTGCCTTGGCGGAGCTCGCCACAAAGACCTTCGGCAGCTCC
GAATCGTCGGCCGTCGACAGCGGCACGGCAACGGCCTCTCCTGACCAGCCCTCCG
ACGACGGCGACGCGGGATCCGACGTTGAGTCGTACTCCTCCATGCCCCCCCTTGA
GGGGGAGCCGGGGGATCCCGATCTCAGCGACGGGTCTTGGTCTACCGTAAGCGA
GGAGGCTAGTGAGGACGTCGTCTGCTGC

SEQ ID NO:12: NS5A gene of HCV adaptive replicon III, where nucleotide change is in lower case and highlighted in bold

TCCGGCTCGTGGCTAAGAGATGTTTGGGATTGGATATGCACGGTGTTGACTGATT
TCAAGACCTGGCTCCAGTCCAAGCTCCTGCCGCGATTGCCGGGAGTCCCCTTCTT
CTCATGTCAACGTGGGTACAAGGGAGTCTGGCGGGGCGACGGCATCATGCAAAC
CACCTGCCCATGTGGAGCACAGATCACCGGACATGTGAAAAACGGTTCCATGAG
GATCGTGGGGCCTAGGACCTGTAGTAACACGTGGCATGGAACATTCCCCATTAAC

GCGTACACCACGGGCCCCTGCACGCCCTCCCCGGCGCCAAATTATTCTAGGGCGC
TGTGGCGGGTGGCTGCTGAGGAGTACGTGGAGGTTACGCGGGTGGGGATTTCC
ACTACGTGACGGGCATGACCACTGACAACGTAAAGTGCCCGTGTCAGGTTCCGGC
CCCCGAATTCTTCACAGAAGTGGATGGGGTGCGGTTGCACAGGTACGCTCCAGCG
TGCAAACCCCTCCTACGGGAGGAGGTCACATTCCTGGTCGGGCTCAATCAATACC
TGGTTGGGTCACAGCTCCCATGCGAGCCCGAACCGGACGTAGCAGTGCTCACTTC
CATGCTCACCGACCCCTCCCACATTACGGCGGAGACGGCTAAGCGTAGGCTGGCC
AGGGGATCTCCCCCC**c**CCTTGGCCAGCTCATCAGCTAGCCAGCTGTCTGCGCCTTC
CTTGAAGGCAACATGCACTACCCGTCATGACTCCCCGGACGCTGACCTCATCGAG
GCCAACCTCCTGTGGCGGCAGGAGATGGGCGGGAACATCACCCGCGTGGAGTCA
GAAAATAAGGTAGTAATTTTGGACTCTTTCGAGCCGCTCCAAGCGGAGGAGGAT
GAGAGGGAAGTATCCGTTCCGGCGGAGATCCTGCGGAGGTCCAGGAAATTCCCT
CGAGCGATGCCCATATGGGCACGCCCGGATTACAACCCTCCACTGTTAGAGTCCT
GGAAGGACCCGGACTACGTCCCTCCAGTGGTACACGGGTGTCCATTGCCGCCTGC
CAAGGCCCCTCCGATACCACCTCCACGGAGGAAGAGGACGGTTGTCCTGTCAGA
ATCTACCGTGTCTTCTGCCTTGGCGGAGCTCGCCACAAAGACCTTCGGCAGCTCC
GAATCGTCGGCCGTCGACAGCGGCACGGCAACGGCCTCTCCTGACCAGCCCTCCG
ACGACGGCGACGCGGGATCCGACGTTGAGTCGTACTCCTCCATGCCCCCCCTTGA
GGGGGAGCCGGGGGATCCCGATCTCAGCGACGGGTCTTGGTCTACCGTAAGCGA
GGAGGCTAGTGAGGACGTCGTCTGCTGC

SEQ ID NO:13: Nucleotide sequence of DNA clone of HCV adaptive replicon VII, where nucleotide change is in lower case and highlighted in bold

GCCAGCCCCCGATTGGGGGCGACACTCCACCATAGATCACTCCCCTGTGAGGAAC
TACTGTCTTCACGCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTGCAG
CCTCCAGGACCCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTA
CACCGGAATTGCCAGGACGACCGGGTCCTTTCTTGGATCAACCCGCTCAATGCCT
GGAGATTTGGGCGTGCCCCCGCGAGACTGCTAGCCGAGTAGTGTTGGGTCGCGA
AAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCCCCGGGAGGTCTCGT
AGACCGTGCACCATGAGCACGAATCCTAAACCTCAAAGAAAAACCAAAGGGCGC
GCCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGA
GGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGT
GTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCC
GGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACG
ACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACT
GGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCC
TGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGAT
CCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGT
ACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAG
GGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGC
GAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAA
ATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTA
TCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGG
GCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGC
CTTCTATCGCCTTCTTGACGAGTTCTTCTGAGTTTAAACAGACCACAACGGTTTCC
CTCTAGCGGGATCAATTCCGCCCCTCTCCCTCCCCCCCCCCTAACGTTACTGGCCG
AAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATAT
TGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAG
CATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTC
GTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCG
ACCCTTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAA

AGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTG
TGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAA
GGGGCTGAAGGATGCCCAGAAGGTACCCCATTGTATGGGATCTGATCTGGGGCCT
CGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCCCC
GAACCACGGGGACGTGGTTTTCCTTTGAAAAACACGATAATACCATGGCGCCTAT
TACGGCCTACTCCCAACAGACGCGAGGCCTACTTGGCTGCATCATCACTAGCCTC
ACAGGCCGGGACAGGAACCAGGTCGAGGGGGAGGTCCAAGTGGTCTCCACCGCA
ACACAATCTTTCCTGGCGACCTGCGTCAATGGCGTGTGTTGGACTGTCTATCATG
GTGCCGGCTCAAAGACCCTTGCCGGCCCAAAGGGCCCAATCACCCAAATGTACA
CCAATGTGGACCAGGACCTCGTCGGCTGGCAAGCGCCCCCCGGGGCGCGTTCCTT
GACACCATGCACCTGCGGCAGCTCGGACCTTTACTTGGTCACGAGGCATGCCGAT
GTCATTCCGGTGCGCCGGCGGGGCGACAGCAGGGGGAGCCTACTCTCCCCCAGG
CCCGTCTCCTACTTGAAGGGCTCTTCGGGCGGTCCACTGCTCTGCCCCTCGGGGC
ACGCTGTGGGCATCTTTCGGGCTGCCGTGTGCACCCGAGGGGTTGCGAAGGCGGT
GGACTTTGTACCCGTCGAGTCTATGGAAACCACTATGCGGTCCCCGGTCTTCACG
GACAACTCGTCCCCTCCGGCCGTACCGCAGACATTCCAGGTGGCCCATCTACACG
CCCCTACTGGTAGCGGCAAGAGCACTAAGGTGCCGGCTGCGTATGCAGCCCAAG
GGTATAAGGTGCTTGTCCTGAACCCGTCCGTCGCCGCCACCCTAGGTTTCGGGGC
GTATATGTCTAAGGCACATGGTATCGACCCTAACATCAGAACCGGGGTAAGGAC
CATCACCACGGGTGCCCCCATCACGTACTCCACCTATGGCAAGTTTCTTGCCGAC
GGTGGTTGCTCTGGGGGCGCCTATGACATCATAATATGTGATGAGTGCCACTCAA
CTGACTCGACCACTATCCTGGGCATCGGCACAGTCCTGGACCAAGCGGAGACGG
CTGGAGCGCGACTCGTCGTGCTCGCCACCGCTACGCCTCCGGGATCGGTCACCGT
GCCACATCCAAACATCGAGGAGGTGGCTCTGTCCAGCACTGGAGAAATCCCCTTT
TATGGCAAAGCCATCCCCATCGAGACCATCAAGGGGGGGAGGCACCTCATTTTCT
GCCATTCCAAGAAGAAATGTGATGAGCTCGCCGCGAAGCTGTCCGGCCTCGGACT
CAATGCTGTAGCATATTACCGGGGCCTTGATGTATCCGTCATACCAACTAGCGGA
GACGTCATTGTCGTAGCAACGGACGCTCTAATGACGGGCTTTACCGGCGATTTCG
ACTCAGTGATCGACTGCAATACATGTGTCACCCAGACAGTCGACTTCAGCCTGGA
CCCGACCTTCACCATTGAGACGACGACCGTGCCACAAGACGCGGTGTCACGCTCG
CAGCGGCGAGGCAGGACTGGTAGGGGCAGGATGGGCATTTACAGGTTTGTGACT
CCAGGAGAACGGCCCTCGGGCATGTTCGATTCCTCGGTTCTGTGCGAGTGCTATG
ACGCGGGCTGTGCTTGGTACGAGCTCACGCCCGCCGAGACCTCAGTTAGGTTGCG
GGCTTACCTAAACACACCAGGGTTGCCCGTCTGCCAGGACCATCTGGAGTTCTGG
GAGAGCGTCTTTACAGGCCTCACCCACATAGACGCCCATTTCTTGTCCCAGACTA
AGCAGGCAGGAGACAACTTCCCCTACCTGGTAGCATACCAGGCTACGGTGTGCG
CCAGGGCTCAGGCTCCACCTCCATCGTGGGACCAAATGTGGAAGTGTCTCATACG
GCTAAAGCCTACGCTGCACGGGCCAACGCCCCTGCTGTATAGGCTGGGAGCCGTT
CAAAACGAGGTTACTACCACACACCCCATAACCAAATACATCATGGCATGCATGT
CGGCTGACCTGGAGGTCGTCACGAGCACCTGGGTGCTGGTAGGCGGAGTCCTAG
CAGCTCTGGCCGCGTATTGCCTGACAACAGGCAGCGTGGTCATTGTGGGCAGGAT
CATCTTGTCCGGAAAGCCGGCCATCATTCCCGACAGGGAAGTCCTTTACCGGGAG
TTCGATGAGATGGAAGAGTGCGCCTCACACCTCCCTTACATCGAACAGGGAATGC
AGCTCGCCGAACAATTCAAACAGAAGGCAATCGGGTTGCTGCAAACAGCCACCA
AGCAAGCGGAGGCTGCTGCTCCCGTGGTGGAATCCAAGTGGCGGACCCTCGAAG
CCTTCTGGGCGAAGCATATGTGGAATTTCATCAGCGGGATACAATATTTAGCAGG
CTTGTCCACTCTGCCTGGCAACCCCGCGATAGCATCACTGATGGCATTCACAGCC
TCTATCACCAGCCCGCTCACCACCCAACATACCCTCCTGTTTAACATCCTGGGGG
GATGGGTGGCCGCCCAACTTGCTCCTCCCAGCGCTGCTTCTGCTTTCGTAGGCGCC
GGCATCGCTGGAGCGGCTGTTGGCAGCATAGGCCTTGGGAAGGTGCTTGTGGATA
TTTTGGCAGGTTATGGAGCAGGGGTGGCAGGCGCGCTCGTGGCCTTTAAGGTCAT
GAGCGGCGAGATGCCCTCCACCGAGGACCTGGTTAACCTACTCCCTGCTATCCTC
TCCCCTGGCGCCCTAGTCGTCGGGGTCGTGTGCGCAGCGATACTGCGTCGGCACG

TGGGCCCAGGGGAGGGGGCTGTGCAGTGGATGAACCGGCTGATAGCGTTCGCTT
CGCGGGGTAACCACGTCTCCCCCACGCACTATGTGCCTGAGAGCGACGCTGCAGC
ACGTGTCACTCAGATCCTCTCTAGTCTTACCATCACTCAGCTGCTGAAGAGGCTTC
ACCAGTGGATCAACGAGGACTGCTCCACGCCATGCTCCGGCTCGTGGCTAAGAG
ATGTTTGGGATTGGATATGCACGGTGTTGACTGATTTCAAGACCTGGCTCCAGTC
CAAGCTCCTGCCGCGATTGCCGGGAGTCCCCTTCTTCTCATGTCAACGTGGGTAC
AAGGGAGTCTGGCGGGGCGACGGCATCATGCAAACCACCTGCCCATGTGGAGCA
CAGATCACCGGACATGTGAAAAACGGTTCCATGAGGATCGTGGGGCCTAGGACC
TGTAGTAACACGTGGCATGGAACATTCCCCATTAACGCGTACACCACGGGCCCCT
GCACGCCCTCCCCGGCGCCAAATTATTCTAGGGCGCTGTGGCGGGTGGCTGCTGA
GGAGTACGTGGAGGTTACGCGGGTGGGGGATTTCCACTACGTGACGGGCATGAC
CACTGACAACGTAAAGTGCCCGTGTCAGGTTCCGGCCCCCGAATTCTTCACAGAA
GTGGATGGGGTGCGGTTGCACAGGTACGCTCCAGCGTGCAAACCCCTCCTACGGG
AGGAGGTCACATTCCTGGTCGGGCTCAATCAATACCTGGTTGGGTCACAGCTCCC
ATGCGAGCCCGAACCGGACGTAGCAGTGCTCACTTCCATGCTCACCGACCCCTCC
CACATTACGGCGGAGACGGCTAAGCGTAGGCTGGCCAGGGGATCTCCCCCCTCCT
TGGCCAGCTCATCAGCTAtCCAGCTGTCTGCGCCTTCCTTGAAGGCAACATGCACT
ACCCGTCATGACTCCCCGGACGCTGACCTCATCGAGGCCAACCTCCTGTGGCGGC
AGGAGATGGGCGGGAACATCACCCGCGTGGAGTCAGAAAATAAGGTAGTAATTT
TGGACTCTTTCGAGCCGCTCCAAGCGGAGGAGGATGAGAGGGAAGTATCCGTTC
CGGCGGAGATCCTGCGGAGGTCCAGGAAATTCCCTCGAGCGATGCCCATATGGG
CACGCCCGGATTACAACCCTCCACTGTTAGAGTCCTGGAAGGACCCGGACTACGT
CCCTCCAGTGGTACACGGGTGTCCATTGCCGCCTGCCAAGGCCCCTCCGATACCA
CCTCCACGGAGGAAGAGGACGGTTGTCCTGTCAGAATCTACCGTGTCTTCTGCCT
TGGCGGAGCTCGCCACAAAGACCTTCGGCAGCTCCGAATCGTCGGCCGTCGACA
GCGGCACGGCAACGGCCTCTCCTGACCAGCCCTCCGACGACGGCGACGCGGGAT
CCGACGTTGAGTCGTACTCCTCCATGCCCCCCCTTGAGGGGGAGCCGGGGGATCC
CGATCTCAGCGACGGGTCTTGGTCTACCGTAAGCGAGGAGGCTAGTGAGGACGT
CGTCTGCTGCTCGATGTCCTACACATGGACAGGCGCCCTGATCACGCCATGCGCT
GCGGAGGAAACCAAGCTGCCCATCAATGCACTGAGCAACTCTTTGCTCCGTCACC
ACAACTTGGTCTATGCTACAACATCTCGCAGCGCAAGCCTGCGGCAGAAGAAGG
TCACCTTTGACAGACTGCAGGTCCTGGACGACCACTACCGGGACGTGCTCAAGGA
GATGAAGGCGAAGGCGTCCACAGTTAAGGCTAAACTTCTATCCGTGGAGGAAGC
CTGTAAGCTGACGCCCCCACATTCGGCCAGATCTAAATTTGGCTATGGGGCAAAG
GACGTCCGGAACCTATCCAGCAAGGCCGTTAACCACATCCGCTCCGTGTGGAAGG
ACTTGCTGGAAGACACTGAGACACCAATTGACACCACCATCATGGCAAAAAATG
AGGTTTTCTGCGTCCAACCAGAGAAGGGGGGCCGCAAGCCAGCTCGCCTTATCGT
ATTCCCAGATTTGGGGGTTCGTGTGTGCGAGAAAATGGCCCTTTACGATGTGGTC
TCCACCCTCCCTCAGGCCGTGATGGGCTCTTCATACGGATTCCAATACTCTCCTGG
ACAGCGGGTCGAGTTCCTGGTGAATGCCTGGAAAGCGAAGAAATGCCCTATGGG
CTTCGCATATGACACCCGCTGTTTTGACTCAACGGTCACTGAGAATGACATCCGT
GTTGAGGAGTCAATCTACCAATGTTGTGACTTGGCCCCCGAAGCCAGACAGGCCA
TAAGGTCGCTCACAGAGCGGCTTTACATCGGGGGCCCCCTGACTAATTCTAAAGG
GCAGAACTGCGGCTATCGCCGGTGCCGCGCGAGCGGTGTACTGACGACCAGCTG
CGGTAATACCCTCACATGTTACTTGAAGGCCGCTGCGGCCTGTCGAGCTGCGAAG
CTCCAGGACTGCACGATGCTCGTATGCGGAGACGACCTTGTCGTTATCTGTGAAA
GCGCGGGGACCCAAGAGGACGAGGCGAGCCTACGGGCCTTCACGGAGGCTATGA
CTAGATACTCTGCCCCCCCTGGGGACCCGCCCAAACCAGAATACGACTTGGAGTT
GATAACATCATGCTCCTCCAATGTGTCAGTCGCGCACGATGCATCTGGCAAAAGG
GTGTACTATCTCACCCGTGACCCCACCACCCCCCTTGCGCGGGCTGCGTGGGAGA
CAGCTAGACACACTCCAGTCAATTCCTGGCTAGGCAACATCATCATGTATGCGCC
CACCTTGTGGGCAAGGATGATCCTGATGACTCATTTCTTCTCCATCCTTCTAGCTC
AGGAACAACTTGAAAAAGCCCTAGATTGTCAGATCTACGGGGCCTGTTACTCCAT

TGAGCCACTTGACCTACCTCAGATCATTCAACGACTCCATGGCCTTAGCGCATTTT
CACTCCATAGTTACTCTCCAGGTGAGATCAATAGGGTGGCTTCATGCCTCAGGAA
ACTTGGGGTACCGCCCTTGCGAGTCTGGAGACATCGGGCCAGAAGTGTCCGCGCT
AGGCTACTGTCCCAGGGGGGGAGGGCTGCCACTTGTGGCAAGTACCTCTTCAACT
GGGCAGTAAGGACCAAGCTCAAACTCACTCCAATCCCGGCTGCGTCCCAGTTGGA
TTTATCCAGCTGGTTCGTTGCTGGTTACAGCGGGGGAGACATATATCACAGCCTG
TCTCGTGCCCGACCCCGCTGGTTCATGTGGTGCCTACTCCTACTTTCTGTAGGGGT
AGGCATCTATCTACTCCCCAACCGATGAACGGGGAGCTAAACACTCCAGGCCAAT
AGGCCATCCTGTTTTTTTCCCTTTTTTTTTTTCTTTTTTTTTTTTTTTTTTTTTTTT
TTTTTCTCCTTTTTTTTTCCTCTTTTTTTCCTTTTCTTTCCTTTGGTGGCTCCATCTTA
GCCCTAGTCACGGCTAGCTGTGAAAGGTCCGTGAGCCGCTTGACTGCAGAGAGTG
CTGATACTGGCCTCTCTGCAGATCAAGT

SEQ ID NO:14: Amino acid sequence of the NS5A protein of HCV adaptive replicon I, where amino acid generated is highlighted in bold

SGSWLRDVWDWICTVLTDFKTWLQSKLLPRLPGVPFFSCQRGYKGVWRGDGIMQTT
CPCGAQITGHVKNGSMRIVGPRTCSNTWHGTFPINAYTTGPCTPSPAPNYSRALWRV
AAEEYVEVTRVGDFHYVTGMTTDNVKCPCQVPAPEFFTEVDGVRLHRYAPACKPLL
REEVTFLVGLNQYLVGSQLPCEPEPDVAVLTSMLTDPSHITAETAKRRLARGSPPSLA
SSSASQL**Y**SFEPLQAEEDEREVSVPAEILRRSRKFPRAMPIWARPDYNPPLLESWKDP
DYVPPVVHGCPLPPAKAPPIPPPRRKRTVVLSESTVSSALAELATKTFGSSESSAVDSG
TATASPDQPSDDGDAGSDVESYSSMPPLEGEPGDPDLSDGSWSTVSEEASEDVVCC

SEQ ID NO:15: Amino acid sequence of the polyprotein coding region of HCV adaptive replicon VI, where amino acid changes are highlighted in bold

MAPITAYSQQTRGLLGCIITSLTGRDRNQVEGEVQVVSTATQSFLATCVNGVCWTVY
HGAGSKTLAGPKGPITQMYTNVDQDLVGW**R**APPGARSLTPCTCGSSDLYLVTRHAD
VIPVRRRGDSRGSLLSPRPVSYLKGSSGGPLLCPSGHAVGIFRAAVCTRGVAKAVDFV
PVESMETTMRSPVFTDNSSPPAVPQTFQVAHLHAPTGSGKSTKVPAAYAAQGYKVL
VLNPSVAATLGFGAYMSKAHGIDPNIRTGVRTITTGAPITYSTYGKFLADGGCSGGAY
DIIICDECHSTDSTTILGIGTVLDQAETAGARLVVLATATPPGSVTVPHPNIEEVALSST
GEIPFYGKAIPIETIKGGRHLIFCHSKKKCDELAAKLSGLGLNAVAYYRGLDVSVIPTS
GDVIVVATDALMTGFTGDFDSVIDCNTCVTQTVDFSLDPTFTIETTTVPQDAVSRSQR
RGRTGRGRMGIYRFVTPGERPSGMFDSSVLCECYDAGCAWYELTPAETSVRLRAYL
NTPGLPVCQDHLEFWESVFTGLTHIDAHFLSQTKQAGDNFPYLVAYQATVCARAQA
PPPSWDQMWKCLIRLKPTLHGPTPLLYRLGAVQNEVTTTHPITKYIMACMSADLEVV
TSTWVLVGGVLAALAAYCLTTGSVVIVGRIILSGKPAIIPDREVLYREFDEMEECASH
LPYIEQGMQLAEQFKQKAIGLLQTATKQAEAAAPVVESKWRTLEAFWAKHMWNFIS
GIQYLAGLSTLPGNPAIASLMAFTASITSPLTTQHTLLFNILGGWVAAQLAPPSAASAF
VGAGIAGAAVGSIGLGKVLVDILAGYGAGVAGALVAFKVMSGEMPSTEDLVNLLPA
ILSPGALVVGVVCAAILRRHVGPGEGAVQWMNRLIAFASRGNHVSPTHYVPESDAA
ARVTQILSSLTITQLLKRLHQWINEDCSTPCSGSWLRDVWDWICTVLTDFKTWLQSK
LLPRLPGVPFFSCQRGYKGVWRGDGIMQTTCPCGAQITGHVKNGSMRIVGPRTCSNT
WHGTFPINAYTTGPCTPSPAPNYSRALWRVAAEEYVEVTRVGDFHYVTGMTTDNVK
CPCQVPAPEFFTEVDGVRLHRYAPACKPLLREEVTFLVGLNQYLVGSQLPCEPEPDV
AVLTSMLTDPSHITAETAKRRLARGSPPSLASSSA**I**QLSAPSLKATCTTRHDSPDADLI
EANLLWRQEMGGNITRVESENKVVILDSFEPLQAEEDEREVSVPAEILRRSRKFPRAM
PIWARPDYNPPLLESWKDPDYVPPVVHGCPLPPAKAPPIPPPRRKRTVVLSESTVSSAL
AELATKTFGSSESSAVDSGTATASPDQPSDDGDAGSDVESYSSMPPLEGEPGDPDLSD

GSWSTVSEEASEDVVCCSMSYTWTGALITPCAAEETKLPINALSNSLLRHHNLVYAT
TSRSASLRQKKVTFDRLQVLDDHYRDVLKEMKAKASTVKAKLLSVEEACKLTPPHS
ARSKFGYGAKDVRNLSSKAVNHIRSVWKDLLEDTETPIDTTIMAKNEVFCVQPEKGG
RKPARLIVFPDLGVRVCEKMALYDVVSTLPQAVMGSSYGFQYSPGQRVEFLVNAWK
AKKCPMGFAYDTRCFDSTVTENDIRVEESIYQCCDLAPEARQAIRSLTERLYIGGPLT
NSKGQNCGYRRCRASGVLTTSCGNTLTCYLKAAAACRAAKLQDCTMLVCGDDLVV
ICESAGTQEDEASLRAFTEAMTRYSAPPGDPPKPEYDLELITSCSSNVSVAHDASGKR
VYYLTRDPTTPLARAAWETARHTPVNSWLGNIIMYAPTLWARMILMTHFFSILLAQE
QLEKALDCQIYGACYSIEPLDLPQIIQRLHGLSAFSLHSYSPGEINRVASCLRKLGVPPL
RVWRHRARSVRARLLSQGGRAATCGKYLFNWAVRTKLKLTPIPAASQLDLSSWFVA
GYSGGDIYHSLSRARPRWFMWCLLLLSVGVGIYLLPNR

SEQ ID NO:16: Amino acid sequence of the NS5A protein of HCV adaptive replicon VII, where amino acid change is highlighted in bold

SGSWLRDVWDWICTVLTDFKTWLQSKLLPRLPGVPFFSCQRGYKGVWRGDGIMQTT
CPCGAQITGHVKNGSMRIVGPRTCSNTWHGTFPINAYTTGPCTPSPAPNYSRALWRV
AAEEYVEVTRVGDFHYVTGMTTDNVKCPCQVPAPEFFTEVDGVRLHRYAPACKPLL
REEVTFLVGLNQYLVGSQLPCEPEPDVAVLTSMLTDPSHITAETAKRRLARGSPPSLA
SSSAIQLSAPSLKATCTTRHDSPDADLIEANLLWRQEMGGNITRVESENKVVILDSFEP
LQAEEDEREVSVPAEILRRSRKFPRAMPIWARPDYNPPLLESWKDPDYVPPVVHGCP
LPPAKAPPIPPPRRKRTVVLSESTVSSALAELATKTFGSSESSAVDSGTATASPDQPSD
DGDAGSDVESYSSMPPLEGEPGDPDLSDGSWSTVSEEASEDVVCC

SEQ ID NO:17: Amino acid sequence of the polyprotein of HCV adaptive replicon II, where amino acid changes are highlighted in bold

MAPITAYSQQTRGLLGCIITSLTGRDRNQVEGEVQVVSTATQSFLATCVNGVCWTVY
HGAGSKTLAGPKGPITQMYTNVDQDLVGWQAPPGARSLTPCTCGSSDLYLVTRHAD
VIPVRRRGDSRGSLLSPRPVSYLKGSSGGPLLCPSGHAVGIFRAAVCTRGVAKAVDFV
PVESMETTMRSPVFTDNSSPPAVPQTFQVAHLHAPTGSGKSTKVPAAYAAQGYKVL
VLNPSVAATLGFGAYMSKAHGIDPNIRTGVRTITTGAPITYSTYGKFLADGGCSGGAY
DIIICDECHSTDSTTILGIGTVLDQAETAGARLVVLATATPPGSVTVPHPNIEEVALSST
GEIPFYGKAIPIETIKGGRHLIFCHSKKKCDELAAKLSGLGLNAVAYYRGLDVSVIPTS
GDVIVVATDALMTGFTGDFDSVIDCNTCVTQTVDFSLDPTFTIETTTVPQDAVSRSQR
RGRTGRGRMGIYRFVTPGERPSGMFDSSVLCECYDAGCAWYELTPAETSVRLRAYL
NTPGLPVCQDHLEFWESVFTGLTHIDAHFLSQTKQAGDNFPYLVAYQATVCARAQA
PPPSWDQMWECLIRLKPTLHGPTPLLYRLGAVQNEVTTTHPITKYIMACMSADLEVV
TSTWVLVGGVLAALAAYCLTTGSVVIVGRIILSGKPAIIPDREVLYREFDEMEECASH
LPYIEQGMQLAEQFKQKAIGLLQTATKQAEAAAPVVESKWRTLEAFWAKHMWNFIS
GIQYLAGLSTLPGNPAIASLMAFTASITSPLTTQHTLLFNILGGWVAAQLAPPSAASAF
VGAGIAGAAVGSIGLGKVLVDILAGYGAGVAGALVAFKVMSGEMPSTEDLVNLLPA
ILSPGALVVGVVCAAILRRHVGPGEGAVQWMNRLIAFASRGNHVSPTHYVPESDAA
ARVTQILSGLTITQLLKRLHQWINEDCSTPCSGSWLRDVWDWICTVLTDFKTWLQSK
LLPRLPGVPFFSCQRGYKGVWRGDGIMQTTCPCGAQITGHVKNGSMRIVGPRTCSNT
WHGTFPINAYTTGPCTPSPAPNYSRALWRVAAEEYVEVTRVGDFHYVTGMTTDNVK
CPCQVPAPEFFTEVDGVRLHRYAPACKPLLREEVTFLVGLNQYLVGSQLPCEPEPDV
AVLTSMLTDPSHITAETAKRGLARGSPPSLASSSASQLSAPSLKATCTTRHDSPDADLI
EANLLWRQEMGGNITRVESENKVVILDSFEPLQAEEDEREVSVPAEILRRSRKFPRAM
PIWARPDYNPPLLESWKDPDYVPPVVHGCPLPPAKAPPIPPPRRKRTVVLSESTVSSAL
AELATKTFGSSESSAVDSGTATASPDQPSDDGDAGSDVESYSSMPPLEGEPGDPDLSD

GSWSTVSEEASEDVVCCSMSYTWTGALITPCAAEETKLPINALSNSLLRHHNLVYAT
TSRSASLRQKKVTFDRLQVLDDHYRDVLKEMKAKASTVKAKLLSVEEACKLTPPHS
ARSKFGYGAKDVRNLSSKAVNHIRSVWKDLLEDTETPIDTTIMAKNEVFCVQPEKGG
RKPARLIVFPDLGVRVCEKMALYDVVSTLPQAVMGSSYGFQYSPGQRVEFLVNAWK
AKKCPMGFAYDTRCFDSTVTENDIRVEESIYQCCDLAPEARQAIRSLTERLYIGGPLT
NSKGQNCGYRRCRASGVLTTSCGNTLTCYLKAAAACRAAKLQDCTMLVCGDDLVV
ICESAGTQEDEASLRAFTEAMTRYSAPPGDPPKPEYDLELITSCSSNVSVAHDASGKR
VYYLTRDPTTPLARAAWETARHTPVNSWLGNIIMYAPTLWARMILMTHFFSILLAQE
QLEKALDCQIYGACYSIEPLDLPQIIQRLHGLSAFSLHSYSPGEINRVASCLRKLGVPPL
RVWRHRARSVRARLLSQGGRAATCGKYLFNWAVRTKLKLTPIPAASQLDLSSWFVA
GYSGGDIYHSLSRARPRWFMWCLLLLSVGVGIYLLPNR

SEQ ID NO:18: Amino acid sequence of the NS5A protein of HCV adaptive replicon II, where amino acid change is highlighted in bold

SGSWLRDVWDWICTVLTDFKTWLQSKLLPRLPGVPFFSCQRGYKGVWRGDGIMQTT
CPCGAQITGHVKNGSMRIVGPRTCSNTWHGTFPINAYTTGPCTPSPAPNYSRALWRV
AAEEYVEVTRVGDFHYVTGMTTDNVKCPCQVPAPEFFTEVDGVRLHRYAPACKPLL
REEVTFLVGLNQYLVGSQLPCEPEPDVAVLTSMLTDPSHITAETAKRGLARGSPPSLA
SSSASQLSAPSLKATCTTRHDSPDADLIEANLLWRQEMGGNITRVESENKVVILDSFE
PLQAEEDEREVSVPAEILRRSRKFPRAMPIWARPDYNPPLLESWKDPDYVPPVVHGCP
LPPAKAPPIPPPRRKRTVVLSESTVSSALAELATKTFGSSESSAVDSGTATASPDQPSD
DGDAGSDVESYSSMPPLEGEPGDPDLSDGSWSTVSEEASEDVVCC

SEQ ID NO:19: Amino acid sequence of the NS5A protein of HCV adaptive replicon V, where amino acid change is highlighted in bold

SGSWLRDVWDWICTVLTDFKTWLQSKLLPRLPGVPFFSCQRGYKGVWRGDGIMQTT
CPCGAQITGHVKNGSMRIVGPRTCSNTWHGTFPINAYTTGPCTPSPAPNYSRALWRV
AAEEYVEVTRVGDFHYVTGMTTDNVKCPCQVPAPEFFTEVDGVRLHRYAPACKPLL
REEVTFLVGLNQYLVGSQLPCEPEPDVAVLTSMLTDPSHITAETAKRRLARGSPPSLS
SSSASQLSAPSLKATCTTRHDSPDADLIEANLLWRQEMGGNITRVESENKVVILDSFE
PLQAEEDEREVSVPAEILRRSRKFPRAMPIWARPDYNPPLLESWKDPDYVPPVVHGCP
LPPAKAPPIPPPRRKRTVVLSESTVSSALAELATKTFGSSESSAVDSGTATASPDQPSD
DGDAGSDVESYSSMPPLEGEPGDPDLSDGSWSTVSEEASEDVVCC

SEQ ID NO:20: Amino acid sequence of the NS5A protein of HCV adaptive replicon IV, where amino acid change is highlighted in bold

SGSWLRDVWDWICTVLTDFKTWLQSKLLPRLPGVPFFSCQRGYKGVWRGDGIMQTT
CPCGAQITGHVKNGSMRIVGPRTCSNTWHGTFPINAYTTGPCTPSPAPNYSRALWRV
AAEEYVEVTRVGDFHYVTGMTTDNVKCPCQVPAPEFFTEVDGVRLHRYAPACKPLL
REEVTFLVGLNQYLVGSQLPCEPEPDVAVLTSMLTDPSHITAETAKRRLARGSPPCLA
SSSASQLSAPSLKATCTTRHDSPDADLIEANLLWRQEMGGNITRVESENKVVILDSFE
PLQAEEDEREVSVPAEILRRSRKFPRAMPIWARPDYNPPLLESWKDPDYVPPVVHGCP
LPPAKAPPIPPPRRKRTVVLSESTVSSALAELATKTFGSSESSAVDSGTATASPDQPSD
DGDAGSDVESYSSMPPLEGEPGDPDLSDGSWSTVSEEASEDVVCC

SEQ ID NO:21: Amino acid sequence of the NS5A protein of HCV adaptive replicon III, where amino acid change is highlighted in bold

SGSWLRDVWDWICTVLTDFKTWLQSKLLPRLPGVPFFSCQRGYKGVWRGDGIMQTT
CPCGAQITGHVKNGSMRIVGPRTCSNTWHGTFPINAYTTGPCTPSPAPNYSRALWRV
AAEEYVEVTRVGDFHYVTGMTTDNVKCPCQVPAPEFFTEVDGVRLHRYAPACKPLL
REEVTFLVGLNQYLVGSQLPCEPEPDVAVLTSMLTDPSHITAETAKRRLARGSPPPLA
SSSASQLSAPSLKATCTTRHDSPDADLIEANLLWRQEMGGNITRVESENKVVILDSFE
PLQAEEDEREVSVPAEILRRSRKFPRAMPIWARPDYNPPLLESWKDPDYVPPVVHGCP
LPPAKAPPIPPPRRKRTVVLSESTVSSALAELATKTFGSSESSAVDSGTATASPDQPSD
DGDAGSDVESYSSMPPLEGEPGDPDLSDGSWSTVSEEASEDVVCC

SEQ ID NO:22: Nucleotide sequence of DNA clone of HCV adaptive replicon HCVrep/NS2-5B (see Figure 9)

GCCAGCCCCCGATTGGGGGCGACACTCCACCATAGATCACTCCCCTGTGAGGAAC
TACTGTCTTCACGCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTGCAG
CCTCCAGGACCCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTA
CACCGGAATTGCCAGGACGACCGGGTCCTTTCTTGGATCAACCCGCTCAATGCCT
GGAGATTTGGGCGTGCCCCCGCGAGACTGCTAGCCGAGTAGTGTTGGGTCGCGA
AAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCCCCGGGAGGTCTCGT
AGACCGTGCACCAGACCACAACGGTTTCCCTCTAGCGGGATCAATTCCGCCCCTC
TCCCTCCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGT
GCGTTTGTCTATATGTTATTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGC
CCGGAAACCTGGCCCTGTCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCCTCTCG
CCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAG
CTTCTTGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCCCCC
ACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATACACCTGC
AAAGGCGGCACAACCCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGT
CAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGT
ACCCCATTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTT
AGTCGAGGTTAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTCCTT
TGAAAAACACGATAATACCATGGACCGGGAGATGGCAGCATCGTGCGGAGGCGC
GGTTTTCGTAGGTCTGATACTCTTGACCTTGTCACCGCACTATAAGCTGTTCCTCG
CTAGGCTCATATGGTGGTTACAATATTTTATCACCAGGGCCGAGGCACACTTGCA
AGTGTGGATCCCCCCCCTCAACGTTCGGGGGGGCCGCGATGCCGTCATCCTCCTC
ACGTGCGCGATCCACCCAGAGCTAATCTTTACCATCACCAAAATCTTGCTCGCCA
TACTCGGTCCACTCATGGTGCTCCAGGCTGGTATAACCAAAGTGCCGTACTTCGT
GCGCGCACACGGGCTCATTCGTGCATGCATGCTGGTGCGGAAGGTTGCTGGGGGT
CATTATGTCCAAATGGCTCTCATGAAGTTGGCCGCACTGACAGGTACGTACGTTT
ATGACCATCTCACCCCACTGCGGGACTGGGCCCACGCGGGCCTACGAGACCTTGC
GGTGGCAGTTGAGCCCGTCGTCTTCTCTGATATGGAGACCAAGGTTATCACCTGG
GGGGCAGACACCGCGGCGTGTGGGGACATCATCTTGGGCCTGCCCGTCTCCGCCC
GCAGGGGGAGGGAGATACATCTGGGACCGGCAGACAGCCTTGAAGGGCAGGGG
TGGCGACTCCTCGCGCCTATTACGGCCTACTCCCAACAGACGCGAGGCCTACTTG
GCTGCATCATCACTAGCCTCACAGGCCGGGACAGGAACCAGGTCGAGGGGGAGG
TCCAAGTGGTCTCCACCGCAACACAATCTTTCCTGGCGACCTGCGTCAATGGCGT
GTGTTGGACTGTCTATCATGGTGCCGGCTCAAAGACCCTTGCCGGCCCAAAGGGC
CCAATCACCCAAATGTACACCAATGTGGACCAGGACCTCGTCGGCTGGCAAGCG
CCCCCCGGGGCGCGTTCCTTGACACCATGCACCTGCGGCAGCTCGGACCTTTACT
TGGTCACGAGGCATGCCGATGTCATTCCGGTGCGCCGGCGGGGCGACAGCAGGG
GGAGCCTACTCTCCCCCAGGCCCGTCTCCTACTTGAAGGGCTCTTCGGGCGGTCC
ACTGCTCTGCCCCTCGGGGCACGCTGTGGGCATCTTTCGGGCTGCCGTGTGCACC

CGAGGGGTTGCGAAGGCGGTGGACTTTGTACCCGTCGAGTCTATGGAAACCACTA
TGCGGTCCCCGGTCTTCACGGACAACTCGTCCCCTCCGGCCGTACCGCAGACATT
CCAGGTGGCCCATCTACACGCCCCTACTGGTAGCGGCAAGAGCACTAAGGTGCC
GGCTGCGTATGCAGCCCAAGGGTATAAGGTGCTTGTCCTGAACCCGTCCGTCGCC
GCCACCCTAGGTTTCGGGGCGTATATGTCTAAGGCACATGGTATCGACCCTAACA
TCAGAACCGGGGTAAGGACCATCACCACGGGTGCCCCCATCACGTACTCCACCTA
TGGCAAGTTTCTTGCCGACGGTGGTTGCTCTGGGGGCGCCTATGACATCATAATA
TGTGATGAGTGCCACTCAACTGACTCGACCACTATCCTGGGCATCGGCACAGTCC
TGGACCAAGCGGAGACGGCTGGAGCGCGACTCGTCGTGCTCGCCACCGCTACGC
CTCCGGGATCGGTCACCGTGCCACATCCAAACATCGAGGAGGTGGCTCTGTCCAG
CACTGGAGAAATCCCCTTTTATGGCAAAGCCATCCCCATCGAGACCATCAAGGGG
GGGAGGCACCTCATTTTCTGCCATTCCAAGAAGAAATGTGATGAGCTCGCCGCGA
AGCTGTCCGGCCTCGGACTCAATGCTGTAGCATATTACCGGGGCCTTGATGTATC
CGTCATACCAACTAGCGGAGACGTCATTGTCGTAGCAACGGACGCTCTAATGACG
GGCTTTACCGGCGATTTCGACTCAGTGATCGACTGCAATACATGTGTCACCCAGA
CAGTCGACTTCAGCCTGGACCCGACCTTCACCATTGAGACGACGACCGTGCCAC
AAGACGCGGTGTCACGCTCGCAGCGGCGAGGCAGGACTGGTAGGGGCAGGATGG
GCATTTACAGGTTTGTGACTCCAGGAGAACGGCCCTCGGGCATGTTCGATTCCTC
GGTTCTGTGCGAGTGCTATGACGCGGGCTGTGCTTGGTACGAGCTCACGCCCGCC
GAGACCTCAGTTAGGTTGCGGGCTTACCTAAACACACCAGGGTTGCCCGTCTGCC
AGGACCATCTGGAGTTCTGGGAGAGCGTCTTTACAGGCCTCACCCACATAGACGC
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TACCAGGCTACGGTGTGCGCCAGGGCTCAGGCTCCACCTCCATCGTGGGACCAAA
TGTGGAAGTGTCTCATACGGCTAAAGCCTACGCTGCACGGGCCAACGCCCCTGCT
GTATAGGCTGGGAGCCGTTCAAAACGAGGTTACTACCACACACCCCATAACCAA
ATACATCATGGCATGCATGTCGGCTGACCTGGAGGTCGTCACGAGCACCTGGGTG
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TGGTCATTGTGGGCAGGATCATCTTGTCCGGAAAGCCGGCCATCATTCCCGACAG
GGAAGTCCTTTACCGGGAGTTCGATGAGATGGAAGAGTGCGCCTCACACCTCCCT
TACATCGAACAGGGAATGCAGCTCGCCGAACAATTCAAACAGAAGGCAATCGGG
TTGCTGCAAACAGCCACCAAGCAAGCGGAGGCTGCTGCTCCCGTGGTGGAATCC
AAGTGGCGGACCCTCGAAGCCTTCTGGGCGAAGCATATGTGGAATTTCATCAGCG
GGATACAATATTTAGCAGGCTTGTCCACTCTGCCTGGCAACCCCGCGATAGCATC
ACTGATGGCATTCACAGCCTCTATCACCAGCCCGCTCACCACCCAACATACCCTC
CTGTTTAACATCCTGGGGGGATGGGTGGCCGCCCAACTTGCTCCTCCCAGCGCT
GCTTCTGCTTTCGTAGGCGCCGGCATCGCTGGAGCGGCTGTTGGCAGCATAGGCC
TTGGGAAGGTGCTTGTGGATATTTTGGCAGGTTATGGAGCAGGGGTGGCAGGCGC
GCTCGTGGCCTTTAAGGTCATGAGCGGCGAGATGCCCTCCACCGAGGACCTGGTT
AACCTACTCCCTGCTATCCTCTCCCCTGGCGCCCTAGTCGTCGGGGTCGTGTGCGC
AGCGATACTGCGTCGGCACGTGGGCCCAGGGGAGGGGGCTGTGCAGTGGATGAA
CCGGCTGATAGCGTTCGCTTCGCGGGGTAACCACGTCTCCCCCACGCACTATGTG
CCTGAGAGCGACGCTGCAGCACGTGTCACTCAGATCCTCTCTAGTCTTACCATCA
CTCAGCTGCTGAAGAGGCTTCACCAGTGGATCAACGAGGACTGCTCCACGCCATG
CTCCGGCTCGTGGCTAAGAGATGTTTGGGATTGGATATGCACGGTGTTGACTGAT
TTCAAGACCTGGCTCCAGTCCAAGCTCCTGCCGCGATTGCCGGGAGTCCCCTTCTT
CTCATGTCAACGTGGGTACAAGGGAGTCTGGCGGGGCGACGGCATCATGCAAAC
CACCTGCCCATGTGGAGCACAGATCACCGGACATGTGAAAAACGGTTCCATGAG
GATCGTGGGGCCTAGGACCTGTAGTAACACGTGGCATGGAACATTCCCCATTAAC
GCGTACACCACGGGCCCCTGCACGCCCTCCCCGGCGCCAAATTATTCTAGGGCGC
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ACTACGTGACGGGCATGACCACTGACAACGTAAAGTGCCCGTGTCAGGTTCC
GGCCCCCGAATTCTTCACAGAAGTGGATGGGGTGCGGTTGCACAGGTACGCTCCA
GCGTGCAAACCCCTCCTACGGGAGGAGGTCACATTCCTGGTCGGGCTCAATCAAT

ACCTGGTTGGGTCACAGCTCCCATGCGAGCCCGAACCGGACGTAGCAGTGCTCAC
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CTTCCTTGAAGGCAACATGCACTACCCGTCATGACTCCCCGGACGCTGACCTCAT
CGAGGCCAACCTCCTGTGGCGGCAGGAGATGGGCGGGAACATCACCCGCGTGGA
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GATGAGAGGGAAGTATCCGTTCCGGCGGAGATCCTGCGGAGGTCCAGGAAATTC
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CCTGGAAGGACCCGGACTACGTCCCTCCAGTGGTACACGGGTGTCCATTGCCGCC
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AACTTCTATCCGTGGAGGAAGCCTGTAAGCTGACGCCCCCACATTCGGCCAGATC
TAAATTTGGCTATGGGGCAAAGGACGTCCGGAACCTATCCAGCAAGGCCGTTAA
CCACATCCGCTCCGTGTGGAAGGACTTGCTGGAAGACACTGAGACACCAATTGAC
ACCACCATCATGGCAAAAAATGAGGTTTTCTGCGTCCAACCAGAGAAGGGGGGC
CGCAAGCCAGCTCGCCTTATCGTATTCCCAGATTTGGGGGTTCGTGTGTGCGAGA
AAATGGCCCTTTACGATGTGGTCTCCACCCTCCCTCAGGCCGTGATGGGCTCTTCA
TACGGATTCCAATACTCTCCTGGACAGCGGGTCGAGTTCCTGGTGAATGCCTGGA
AAGCGAAGAAATGCCCTATGGGCTTCGCATATGACACCCGCTGTTTTGACTCAAC
GGTCACTGAGAATGACATCCGTGTTGAGGAGTCAATCTACCAATGTTGTGACTTG
GCCCCCGAAGCCAGACAGGCCATAAGGTCGCTCACAGAGCGGCTTTACATCGGG
GGCCCCCTGACTAATTCTAAAGGGCAGAACTGCGGCTATCGCCGGTGCCGCGCGA
GCGGTGTACTGACGACCAGCTGCGGTAATACCCTCACATGTTACTTGAAGGCCGC
TGCGGCCTGTCGAGCTGCGAAGCTCCAGGACTGCACGATGCTCGTATGCGGAGAC
GACCTTGTCGTTATCTGTGAAAGCGCGGGGACCCAAGAGGACGAGGCGAGCCTA
CGGGCCTTCACGGAGGCTATGACTAGATACTCTGCCCCCCCTGGGGACCCGCCCA
AACCAGAATACGACTTGGAGTTGATAACATCATGCTCCTCCAATGTGTCAGTCGC
GCACGATGCATCTGGCAAAAGGGTGTACTATCTCACCCGTGACCCCACCACCCCC
CTTGCGCGGGCTGCGTGGGAGACAGCTAGACACACTCCAGTCAATTCCTGGCTAG
GCAACATCATCATGTATGCGCCCACCTTGTGGGCAAGGATGATCCTGATGACTCA
TTTCTTCTCCATCCTTCTAGCTCAGGAACAACTTGAAAAAGCCCTAGATTGTCAGA
TCTACGGGGCCTGTTACTCCATTGAGCCACTTGACCTACCTCAGATCATTCAACG
ACTCCATGGCCTTAGCGCATTTTCACTCCATAGTTACTCTCCAGGTGAGATCAATA
GGGTGGCTTCATGCCTCAGGAAACTTGGGGTACCGCCCTTGCGAGTCTGGAGACA
TCGGGCCAGAAGTGTCCGCGCTAGGCTACTGTCCCAGGGGGGGAGGGCTGCCAC
TTGTGGCAAGTACCTCTTCAACTGGGCAGTAAGGACCAAGCTCAAACTCACTCCA
ATCCCGGCTGCGTCCCAGTTGGATTTATCCAGCTGGTTCGTTGCTGGTTACAGCGG
GGGAGACATATATCACAGCCTGTCTCGTGCCCGACCCCGCTGGTTCATGTGGTGC
CTACTCCTACTTTCTGTAGGGGTAGGCATCTATCTACTCCCCAACCGATGAACGG
GGACCTAAACACTCCAGGCCAATAGGCCATCCTGTTTTTTTCCCTTTTTTTTTTTTCT
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCTCCTTTTTTTTTTCCTCTTTTTTTTCCTT
TTCTTTCCTTTGGTGGCTCCATCTTAGCCCTAGTCACGGCTAGCTGTGAAAGGTCC
GTGAGCCGCTTGACTGCAGAGAGTGCTGATACTGGCCTCTCTGCAGATCAAGT

SEQ ID NO:23: Nucleotide sequence of full-length HCV cDNA clone containing the mutation that results in Ser to Ile at position 1179 of SEQ ID NO:3, and where the 5' NTR is fused to the neomycin phosphotransferase gene and the EMCV IRES is inserted upstream of the HCV open reading frame (see Figure 9)

GCCAGCCCCCGATTGGGGGCGACACTCCACCATAGATCACTCCCCTGTGAGGAAC
TACTGTCTTCACGCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTGCAG
CCTCCAGGACCCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTA
CACCGGAATTGCCAGGACGACCGGGTCCTTTCTTGGATCAACCCGCTCAATGCCT
GGAGATTTGGGCGTGCCCCCGCGAGACTGCTAGCCGAGTAGTGTTGGGTCGCGA
AAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCCCCGGGAGGTCTCGT
AGACCGTGCACCATGAGCACGAATCCTAAACCTCAAAGAAAAACCAAAGGGCGC
GCCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGA
GGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGT
GTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCC
GGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACG
ACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACT
GGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCC
TGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGAT
CCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGT
ACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAG
GGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGC
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CTTCTATCGCCTTCTTGACGAGTTCTTCTGAGTTTAAACAGACCACAACGGTTTCC
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GTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCG
ACCCTTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAA
AGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTG
TGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAA
GGGGCTGAAGGATGCCCAGAAGGTACCCCATTGTATGGGATCTGATCTGGGGCCT
CGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCCCC
GAACCACGGGGACGTGGTTTTCCTTTGAAAAACACGATAATAATGAGCACGAAT
CCTAAACCTCAAAGAAAAACCAAACGTAACACCAACCGCCGCCCACAGGACGTC
AAGTTCCCGGGCGGTGGTCAGATCGTCGGTGGAGTTTACCTGTTGCCGCGCAGGG
GCCCCAGGTTGGGTGTGCGCGCGACTAGGAAGACTTCCGAGCGGTCGCAACCTC
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CCTGGCGCATGGCGTCCGGGTTCTGGAGGACGGCGTGAACTATGCAACAGGGAA
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ACCGTATGGCTTGGGATATGATGATGAACTGGTCACCTACAGCAGCCCTAGTGGT
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GGGGACGATGGCCAAAAACACCCTCGGGATTACGTCCCTCTTTTCACCCGGGTCA
TCCCAGAAAATCCAGCTTGTAAACACCAACGGCAGCTGGCACATCAACAGGACT
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GCCCGCTGCTGCTGTCTACAACGGAGTGGCAGGTATTGCCCTGTTCCTTCACCAC
CCTACCGGCTCTGTCCACTGGTTTGATCCATCTCCATCAGAACGTCGTGGACGTAC
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TGTCCTGTTGCTCTTCCTTCTTCTGGCGGACGCGCGCGTCTGTGCCTGCTTGTGGA
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AGGCTGGTATAACCAAAGTGCCGTACTTCGTGCGCGCACACGGGCTCATTCGTGC
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CATGCACCTGCGGCAGCTCGGACCTTTACTTGGTCACGAGGCATGCCGATGTCAT
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GTGGGCATCTTTCGGGCTGCCGTGTGCACCCGAGGGGTTGCGAAGGCGGTGGACT
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CTCGTCCCCTCCGGCCGTACCGCAGACATTCCAGGTGGCCCATCTACACGCCCCT
ACTGGTAGCGGCAAGAGCACTAAGGTGCCGGCTGCGTATGCAGCCCAAGGGTAT

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ATCCAAACATCGAGGAGGTGGCTCTGTCCAGCACTGGAGAAATCCCCTTTTATGG
CAAAGCCATCCCCATCGAGACCATCAAGGGGGGGAGGCACCTCATTTTCTGCCAT
TCCAAGAAGAAATGTGATGAGCTCGCCGCGAAGCTGTCCGGCCTCGGACTCAAT
GCTGTAGCATATTACCGGGGCCTTGATGTATCCGTCATACCAACTAGCGGAGACG
TCATTGTCGTAGCAACGGACGCTCTAATGACGGGCTTTACCGGCGATTTCGACTC
AGTGATCGACTGCAATACATGTGTCACCCAGACAGTCGACTTCAGCCTGGACCCG
ACCTTCACCATTGAGACGACGACCGTGCCACAAGACGCGGTGTCACGCTCGCAGC
GGCGAGGCAGGACTGGTAGGGGCAGGATGGGCATTTACAGGTTTGTGACTCCAG
GAGAACGGCCCTCGGGCATGTTCGATTCCTCGGTTCTGTGCGAGTGCTATGACGC
GGGCTGTGCTTGGTACGAGCTCACGCCCGCCGAGACCTCAGTTAGGTTGCGGGCT
TACCTAAACACACCAGGGTTGCCCGTCTGCCAGGACCATCTGGAGTTCTGGGAGA
GCGTCTTTACAGGCCTCACCCACATAGACGCCCATTTCTTGTCCCAGACTAAGCA
GGCAGGAGACAACTTCCCCTACCTGGTAGCATACCAGGCTACGGTGTGCGCCAG
GGCTCAGGCTCCACCTCCATCGTGGGACCAAATGTGGAAGTGTCTCATACGGCTA
AAGCCTACGCTGCACGGGCCAACGCCCCTGCTGTATAGGCTGGGAGCCGTTCAAA
ACGAGGTTACTACCACACACCCCATAACCAAATACATCATGGCATGCATGTCGGC
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CTGGCCGCGTATTGCCTGACAACAGGCAGCGTGGTCATTGTGGGCAGGATCATCT
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TCACATTCCTGGTCGGGCTCAATCAATACCTGGTTGGGTCACAGCTCCCATGCGA
GCCCGAACCGGACGTAGCAGTGCTCACTTCCATGCTCACCGACCCCTCCCACATT
ACGGCGGAGACGGCTAAGCGTAGGCTGGCCAGGGGATCTCCCCCCTCCTTGGCC
AGCTCATCAGCTATCCAGCTGTCTGCGCCTTCCTTGAAGGCAACATGCACTACCC

GTCATGACTCCCCGGACGCTGACCTCATCGAGGCCAACCTCCTGTGGCGGCAGGA
GATGGGCGGGAACATCACCCGCGTGGAGTCAGAAAATAAGGTAGTAATTTTGGA
CTCTTTCGAGCCGCTCCAAGCGGAGGAGGATGAGAGGGAAGTATCCGTTCCGGC
GGAGATCCTGCGGAGGTCCAGGAAATTCCCTCGAGCGATGCCCATATGGGCACG
CCCGGATTACAACCCTCCACTGTTAGAGTCCTGGAAGGACCCGGACTACGTCCCT
CCAGTGGTACACGGGTGTCCATTGCCGCCTGCCAAGGCCCCTCCGATACCACCTC
CACGGAGGAAGAGGACGGTTGTCCTGTCAGAATCTACCGTGTCTTCTGCCTTGGC
GGAGCTCGCCACAAAGACCTTCGGCAGCTCCGAATCGTCGGCCGTCGACAGCGG
CACGGCAACGGCCTCTCCTGACCAGCCCTCCGACGACGGCGACGCGGGATCCGA
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CTCAGCGACGGGTCTTGGTCTACCGTAAGCGAGGAGGCTAGTGAGGACGTCGTCT
GCTGCTCGATGTCCTACACATGGACAGGCGCCCTGATCACGCCATGCGCTGCGGA
GGAAACCAAGCTGCCCATCAATGCACTGAGCAACTCTTTGCTCCGTCACCACAAC
TTGGTCTATGCTACAACATCTCGCAGCGCAAGCCTGCGGCAGAAGAAGGTCACCT
TTGACAGACTGCAGGTCCTGGACGACCACTACCGGGACGTGCTCAAGGAGATGA
AGGCGAAGGCGTCCACAGTTAAGGCTAAACTTCTATCCGTGGAGGAAGCCTGTA
AGCTGACGCCCCCACATTCGGCCAGATCTAAATTTGGCTATGGGGCAAAGGACGT
CCGGAACCTATCCAGCAAGGCCGTTAACCACATCCGCTCCGTGTGGAAGGACTTG
CTGGAAGACACTGAGACACCAATTGACACCACCATCATGGCAAAAAATGAGGTT
TTCTGCGTCCAACCAGAGAAGGGGGGCCGCAAGCCAGCTCGCCTTATCGTATTCC
CAGATTTGGGGGTTCGTGTGTGCGAGAAAATGGCCCTTTACGATGTGGTCTCCAC
CCTCCCTCAGGCCGTGATGGGCTCTTCATACGGATTCCAATACTCTCCTGGACAG
CGGGTCGAGTTCCTGGTGAATGCCTGGAAAGCGAAGAAATGCCCTATGGGCTTCG
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GGAGTCAATCTACCAATGTTGTGACTTGGCCCCCGAAGCCAGACAGGCCATAAG
GTCGCTCACAGAGCGGCTTTACATCGGGGGCCCCCTGACTAATTCTAAAGGGCAG
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AATACCCTCACATGTTACTTGAAGGCCGCTGCGGCCTGTCGAGCTGCGAAGCTCC
AGGACTGCACGATGCTCGTATGCGGAGACGACCTTGTCGTTATCTGTGAAAGCGC
GGGGACCCAAGAGGACGAGGCGAGCCTACGGGCCTTCACGGAGGCTATGACTAG
ATACTCTGCCCCCCCTGGGGACCCGCCCAAACCAGAATACGACTTGGAGTTGATA
ACATCATGCTCCTCCAATGTGTCAGTCGCGCACGATGCATCTGGCAAAAGGGTGT
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CAGTAAGGACCAAGCTCAAACTCACTCCAATCCCGGCTGCGTCCCAGTTGGATTT
ATCCAGCTGGTTCGTTGCTGGTTACAGCGGGGGAGACATATATCACAGCCTGTCT
CGTGCCCGACCCCGCTGGTTCATGTGGTGCCTACTCCTACTTTCTGTAGGGGTAGG
CATCTATCTACTCCCCAACCGATGAACGGGGACCTAAACACTCCAGGCCAATAGG
CCATCCTGTTTTTTTCCCTTTTTTTTTTTCTTTTTTTTTTTTTTTTTTTTTTTTTTT
TTTTCTCCTTTTTTTTTTCCTCTTTTTTTCCTTTTCTTTCCTTTGGTGGCTCCATCTTAG
CCCTAGTCACGGCTAGCTGTGAAAGGTCCGTGAGCCGCTTGACTGCAGAGAGTGC
TGATACTGGCCTCTCTGCAGATCAAGT

SEQ ID NO:24: Nucleotide sequence of full-length HCV cDNA clone containing the mutation that results in Ser to Ile at position 1179 of SEQ ID NO:3 (see Figure 9)

GCCAGCCCCCGATTGGGGGCGACACTCCACCATAGATCACTCCCCTGTGAGGAAC
TACTGTCTTCACGCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTGCAG
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CACCGGAATTGCCAGGACGACCGGGTCCTTTCTTGGATCAACCCGCTCAATGCCT
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AAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCCCCGGGAGGTCTCGT
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<u>SEQ ID NO:25:</u> Nucleotide sequence of DNA clone of HCV adaptive replicon 5'NTR-EMCV/HCVrepVII

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GT

What is claimed is:

1. A polynucleotide comprising a non-naturally occurring HCV sequence that is capable of productive replication in a host cell, or is capable of being transcribed into a non-naturally occurring HCV sequence that is capable of productive replication in a host cell, wherein the HCV sequence comprises, from 5′ to 3′ on the positive-sense nucleic acid, a functional 5′ non-translated region (5' NTR); one or more protein coding regions, including at least one polyprotein coding region that is capable of replicating HCV RNA; and a functional HCV 3′ non-translated region (3' NTR).

2. The polynucleotide of claim 1, further comprising an adaptive mutation.

3. The polynucleotide of claim 2, having a transfection efficiency into mammalian cells of greater than 0.01%.

4. The polynucleotide of claim 3, wherein the transfection efficiency into mammalian cells is greater than 0.1%.

5. The polynucleotide of claim 3, wherein the transfection efficiency into mammalian cells is greater than 1%.

6. The polynucleotide of claim 3, wherein the transfection efficiency into mammalian cells is greater than 5%.

7. The polynucleotide of claim 2, wherein the polynucleotide is capable of replication in a non-hepatic cell.

8. The polynucleotide of claim 7, wherein the non-hepatic cell is a HeLa cell.

9. The polynucleotide of claim 2, wherein the HCV is impaired in its ability to cause disease, establish chronic infections, trigger autoimmune responses, and transform cells.

10. The polynucleotide of claim 2, wherein the polyprotein region comprises an NS5A gene that is not a wild-type NS5A gene.

11. The polynucleotide of claim 10, wherein the NS5A gene comprises a mutation.

12. The polynucleotide of claim 11, wherein the mutation is within 50 nucleotides of an ISDR or includes the ISDR.

13. The polynucleotide of claim 12, wherein the mutation is within 20 nt of the ISDR, or includes the ISDR.

14. The polynucleotide of claim 13, wherein the mutation encodes an amino acid sequence change selected from the group consisting of Ser (1179) to Ile, Arg (1164) to Gly, Ala(1174) to Ser, Ser(1172) to Cys, and Ser(1172) to Pro of SEQ ID NO:3.

15. The polynucleotide of claim 11, wherein the mutation comprises a deletion of at least a portion of the ISDR.

16. The polynucleotide of claim 15, wherein the mutation comprises a deletion of the entire ISDR.

17. The polynucleotide of claim 16, wherein the mutation comprises a deletion of nucleotides corresponding to nucleotides 5345 to 5485 of SEQ ID NO:6.

18. The polynucleotide of claim 1, wherein the polynucleotide comprises at least one IRES selected from the group consisting of a viral IRES, a cellular IRES, and an artificial IRES.

19. The polynucleotide of claim 18, wherein the HCV polyprotein coding region encodes all HCV structural and nonstructural proteins.

20. The polynucleotide of claim 19, further comprising a foreign gene operably linked to a first IRES and the HCV polyprotein coding region operably linked to a second IRES.

21. The polynucleotide of claim 18, wherein the polyprotein coding region is incapable of making infectious HCV particles.

22. The polynucleotide of claim 21, wherein the polyprotein coding region comprises a mutation and/or a deletion in the structural protein coding region.

23. The polynucleotide of claim 22, further comprising a foreign gene operably linked to a first IRES and the HCV polyprotein coding region operably linked to a second IRES.

24. The polynucleotide of claim 23, wherein the foreign gene is a gene encoding a selectable marker or a reporter gene.

25. The polynucleotide of claim 24, further comprising an adaptive mutation.

26. The polynucleotide of claim 25, having a transfection efficiency into mammalian cells of greater than 0.01%.

27. The polynucleotide of claim 26, wherein the transfection efficiency into mammalian cells is greater than 1%.

28. The polynucleotide of claim 26, wherein the transfection efficiency into mammalian cells is greater than 5%.

29. The polynucleotide of claim 26, wherein the transfection efficiency into mammalian cells is about 6%.

30. The polynucleotide of claim 25, wherein the polynucleotide is capable of replication in a non-hepatic cell.

31. The polynucleotide of claim 30, wherein the non-hepatic cell is a HeLa cell.

32. The polynucleotide of claim 25, wherein the HCV is impaired in its ability to cause disease, establish chronic infections, trigger autoimmune responses, and transform cells.

33. The polynucleotide of claim 25, wherein the polyprotein region comprises an NS5A gene that is not a wild-type NS5A gene.

34. The polynucleotide of claim 33, wherein the NS5A gene comprises a mutation.

35. The polynucleotide of claim 34, wherein the mutation is within 50 nucleotides of an ISDR or includes the ISDR.

36. The polynucleotide of claim 34, wherein the mutation is within 20 nt of the ISDR, or includes the ISDR.

37. The polynucleotide of claim 36, wherein the mutation encodes an amino acid sequence change selected from the group consisting of Ser (1179) to Ile, Arg (1164) to Gly, Ala(1174) to Ser, Ser(1172) to Cys, and Ser(1172) to Pro of SEQ ID NO:3.

38. The polynucleotide of claim 34, wherein the mutation comprises a deletion of at least a portion of the ISDR.

39. The polynucleotide of claim 38, wherein the mutation comprises a deletion of the entire ISDR.

40. The polynucleotide of claim 39, wherein the mutation comprises a deletion of nucleotides corresponding to nucleotides 5345 to 5485 of SEQ ID NO:6.

41. The polynucleotide of claim 24, wherein:

(a) the first IRES is an HCV IRES;

(b) the foreign gene is a *neo* gene; and

(c) the second IRES is a EMCV IRES.

42. The polynucleotide of claim 41, wherein the HCV sequence is a genotype 1 HCV sequence.

43. The polynucleotide of claim 42, wherein the HCV sequence is subtype 1b.

44. The polynucleotide of claim 41, comprising SEQ ID NO:5 or SEQ ID NO:6.

45. The polynucleotide of claim 41, further comprising an adaptive mutation.

46. The polynucleotide of claim 45, having a transfection efficiency into mammalian cells of greater than 0.01%.

47. The polynucleotide of claim 46, wherein the transfection efficiency into mammalian cells is greater than 1%.

48. The polynucleotide of claim 46, wherein the transfection efficiency into mammalian cells is greater than 5%.

49. The polynucleotide of claim 46, wherein the transfection efficiency into mammalian cells is about 6%.

50. The polynucleotide of claim 45, wherein the polynucleotide is capable of replication in a non-hepatic cell.

51. The polynucleotide of claim 50, wherein the non-hepatic cell is a HeLa cell.

52. The polynucleotide of claim 45, wherein the HCV is impaired in its ability to cause disease, establish chronic infections, trigger autoimmune responses, and transform cells.

53. The polynucleotide of claim 45, wherein the polyprotein region comprises an NS5A gene that is not a wild-type NS5A gene.

54. The polynucleotide of claim 53, wherein the NS5A gene comprises a mutation.

55. The polynucleotide of claim 54, wherein the mutation is within 50 nucleotides of an ISDR or includes the ISDR.

56. The polynucleotide of claim 54, wherein the mutation is within 20 nt of the ISDR, or includes the ISDR.

57. The polynucleotide of claim 56, wherein the mutation encodes an amino acid sequence change selected from the group consisting of Ser (1179) to Ile, Arg (1164) to Gly, Ala(1174) to Ser, Ser(1172) to Cys, and Ser(1172) to Pro of SEQ ID NO:3.

58. The polynucleotide of claim 54, wherein the mutation comprises a deletion of at least a portion of the ISDR.

59. The polynucleotide of claim 58, wherein the mutation comprises a deletion of the entire ISDR.

60. The polynucleotide of claim 59, wherein the mutation comprises a deletion of nucleotides corresponding to nucleotides 5345 to 5485 of SEQ ID NO:6.

61. The polynucleotide of claim 1, wherein the polynucleotide is double-stranded DNA.

62. A vector comprising the polynucleotide of claim 61 operably associated with a promoter.

63. The polynucleotide of claim 41 wherein the polynucleotide is double-stranded DNA.

64. A vector comprising the polynucleotide of claim 63 operably associated with a promoter.

65. The vector of claim 64, further comprising a mutation in the NS5A gene.

66. The vector of claim 65, wherein the mutation is selected from the group consisting of mutations encoding the amino acid changes Ser (1179) to Ile, Arg (1164) to Gly, Ala(1174) to Ser, Ser(1172) to Cys, and Ser(1172) to Pro of SEQ ID NO:3; and an in frame deletion of nucleotides encoding amino acids comprising at least a portion of the ISDR.

67. The vector of claim 66, wherein the mutation comprises a deletion of the entire ISDR.

68. The vector of claim 67, wherein the mutation comprises a deletion of nucleotides corresponding to nucleotides 5345 to 5485 of SEQ ID NO:6.

69. A cell comprising the vector of claim 62.

70. A host cell comprising the polynucleotide of claim 2, wherein the host cell is a mammalian cell.

71. The host cell of claim 70, wherein the polynucleotide comprises an adaptive mutation.

72. The host cell of claim 71 wherein the host cell is a human cell.

73. The host cell of claim 72 wherein the host cell is a liver cell.

74. The host cell of claim 72 wherein the host cell is a T-cell or a B-cell.

75. The host cell of claim 72 wherein the host cell is a HeLa cell.

76. A method for identifying a cell line that is permissive for infection with HCV, comprising contacting a cell in tissue culture with an infectious amount of the polynucleotide of claim 1, and detecting replication of HCV in cells of the cell line.

77. A method for producing a cell line comprising replicating HCV, the method comprising

(a) transcribing the vector of claim 62 to synthesize HCV RNA;
(b) transfecting a cell with the HCV RNA of step (a); and
(c) culturing the cell.

78. A vaccine comprising the polynucleotide of claim 1 in a pharmaceutically acceptable carrier.

79. The vaccine of claim 78, wherein the polynucleotide further comprises an adaptive mutation.

80. The vaccine of claim 79, wherein the adaptive mutation comprises a deletion of nucleotides corresponding to nucleotides 5345 to 5485 of SEQ ID NO:6.

81. The vaccine of claim 80, wherein the HCV is impaired in its ability to cause disease, establish chronic infections, trigger autoimmune responses, and transform cells.

82. A method of inducing immunoprotection to HCV in a primate, comprising administering to the primate the vaccine of claim 78.

83. A method of inducing immunoprotection to HCV in a primate, comprising administering to the primate the vaccine of claim 81.

84. A method of testing a compound for inhibiting HCV replication, comprising

(a) treating the host cell of claim 70 with the compound;

(b) evaluating the treated host cell for reduced HCV replication, wherein reduced HCV replication indicates the ability of the compound to inhibit HCV replication.

85. A method of testing a compound for inhibiting HCV infection comprising treating a host cell with the compound before, during or after infecting or transfecting the host cell with the polynucleotide of claim 1.

HCV VARIANTS

Table 1. Relative G418 transduction efficiencies of HCV replicons after transfection into interferon-treated cell clones

	Transfected replicon		
Cell line	BartMan	I	VII
parental Huh-7	0.0005%	0.15%	9%
IFN-treated I	0.005%	5%	30%
IFN-treated II	0.001%	1.3%	11%

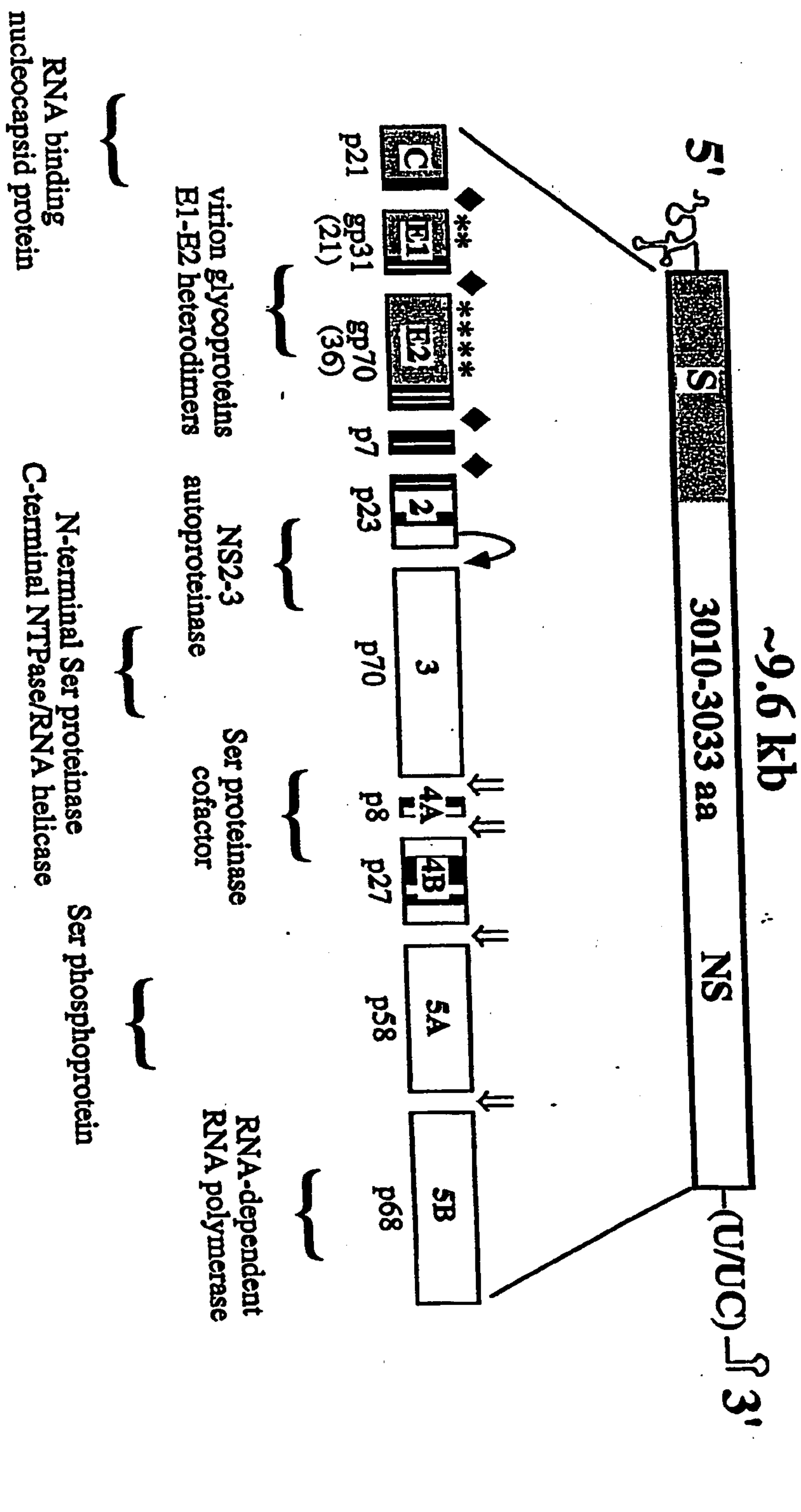
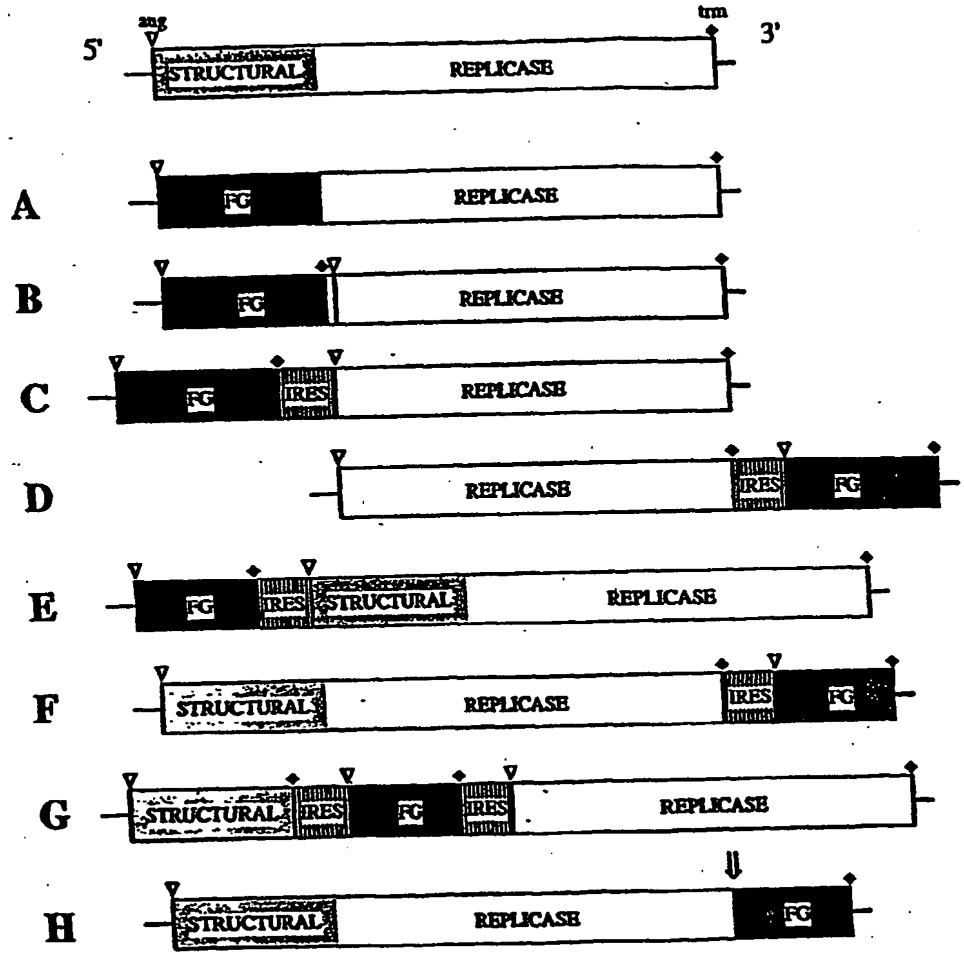

5'
~9.6 kb
3010-3033 aa
S
NS
(U/UC)
3'
C
E1
E2
2
3
4A
4B
5A
5B
p21
gp31
(21)
gp70
(36)
p7
p23
p70
p8
p27
p58
p68
RNA binding
nucleocapsid protein
virion glycoproteins
E1-E2 heterodimers
NS2-3
autoproteinase
N-terminal Ser proteinase
C-terminal NTPase/RNA helicase
Ser proteinase
cofactor
Ser phosphoprotein
RNA-dependent
RNA polymerase

FIGURE 1


aug
trm
5'
3'
STRUCTURAL
REPLICASE
A
FG
REPLICASE
B
FG
REPLICASE
C
FG
IRES
REPLICASE
D
REPLICASE
IRES
FG
E
FG
IRES
STRUCTURAL
REPLICASE
F
STRUCTURAL
REPLICASE
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FG
G
STRUCTURAL
IRES
FG
IRES
REPLICASE
H
STRUCTURAL
REPLICASE
FG

Figure 2

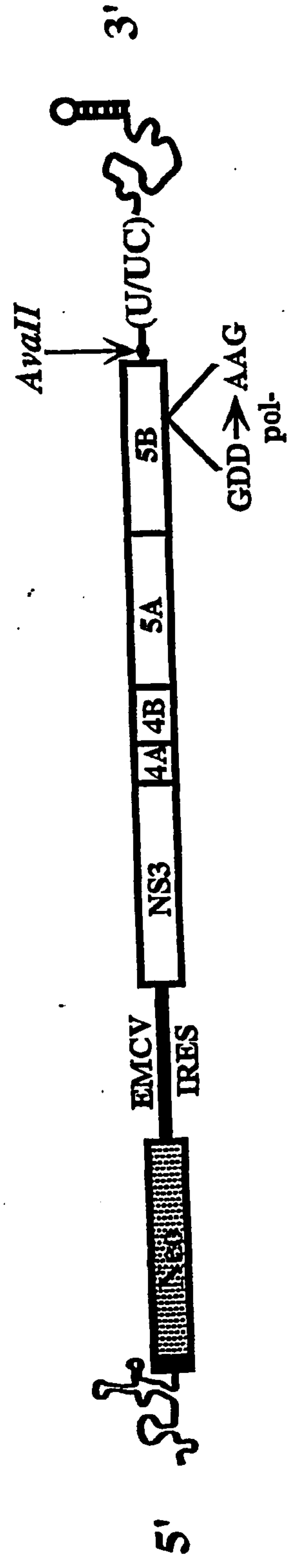
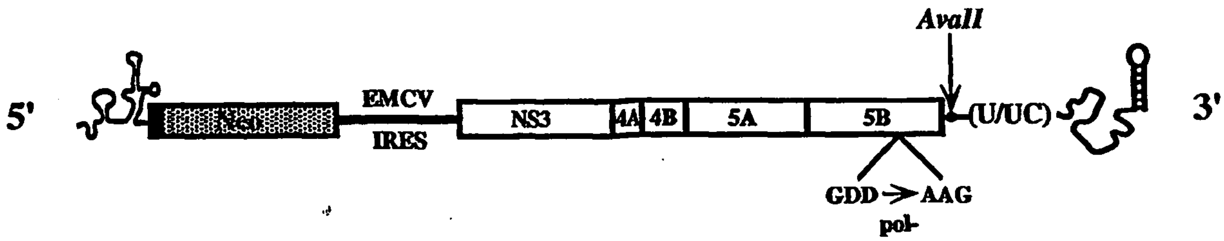


Figure 3

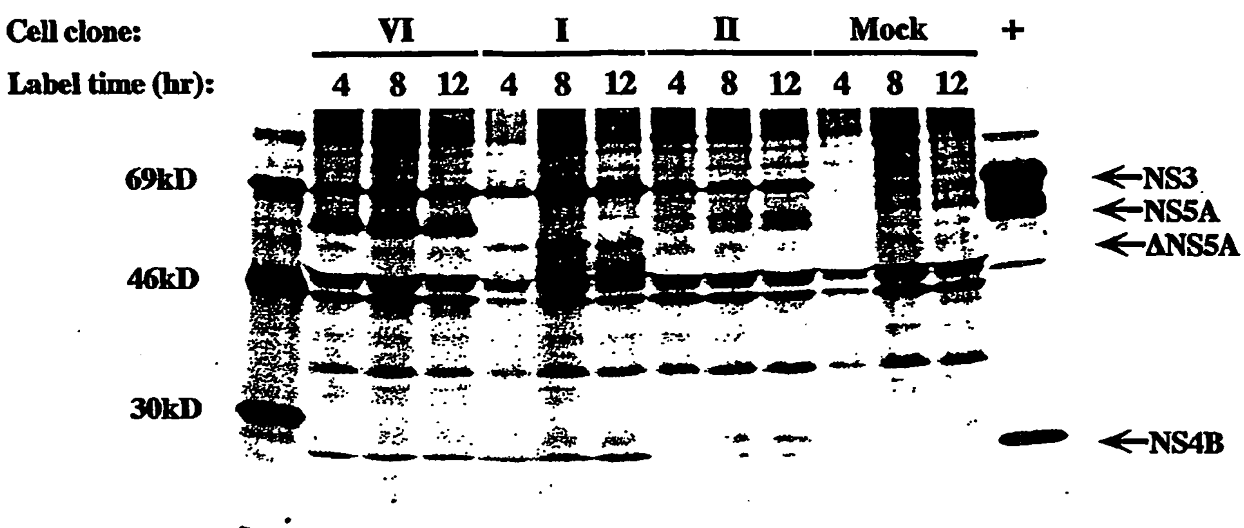


- DNase digest RNA transcripts
- Electroporate RNA into Huh7 cells
- G418-resistant colonies were generated at low frequency
- 28 colonies were picked & 90% of these could be passaged
- No colonies observed for the replicon RNA containing an inactive RDRP

Clone	Copy number/cell	Cytoplasmic NS3	Growth Rate
I	>1000	Yes	Fast
II	~1000-5000	Yes	Fast
IV	ND	Yes	Fast
V	500	ND	Moderate
VI	~1000	Yes	Fast
VII	>800	Yes	Fast
Clone E	<400	No	Very slow

Figure 4

A.


Cell clone:
VI
I
II
Mock
+
Label time (hr):
4 8 12 4 8 12 4 8 12 4 8 12
69kD
46kD
30kD
NS3
NS5A
ΔNS5A
NS4B

B.


Cell clone:
M VI VII I II
HCV RNA

Figure 5

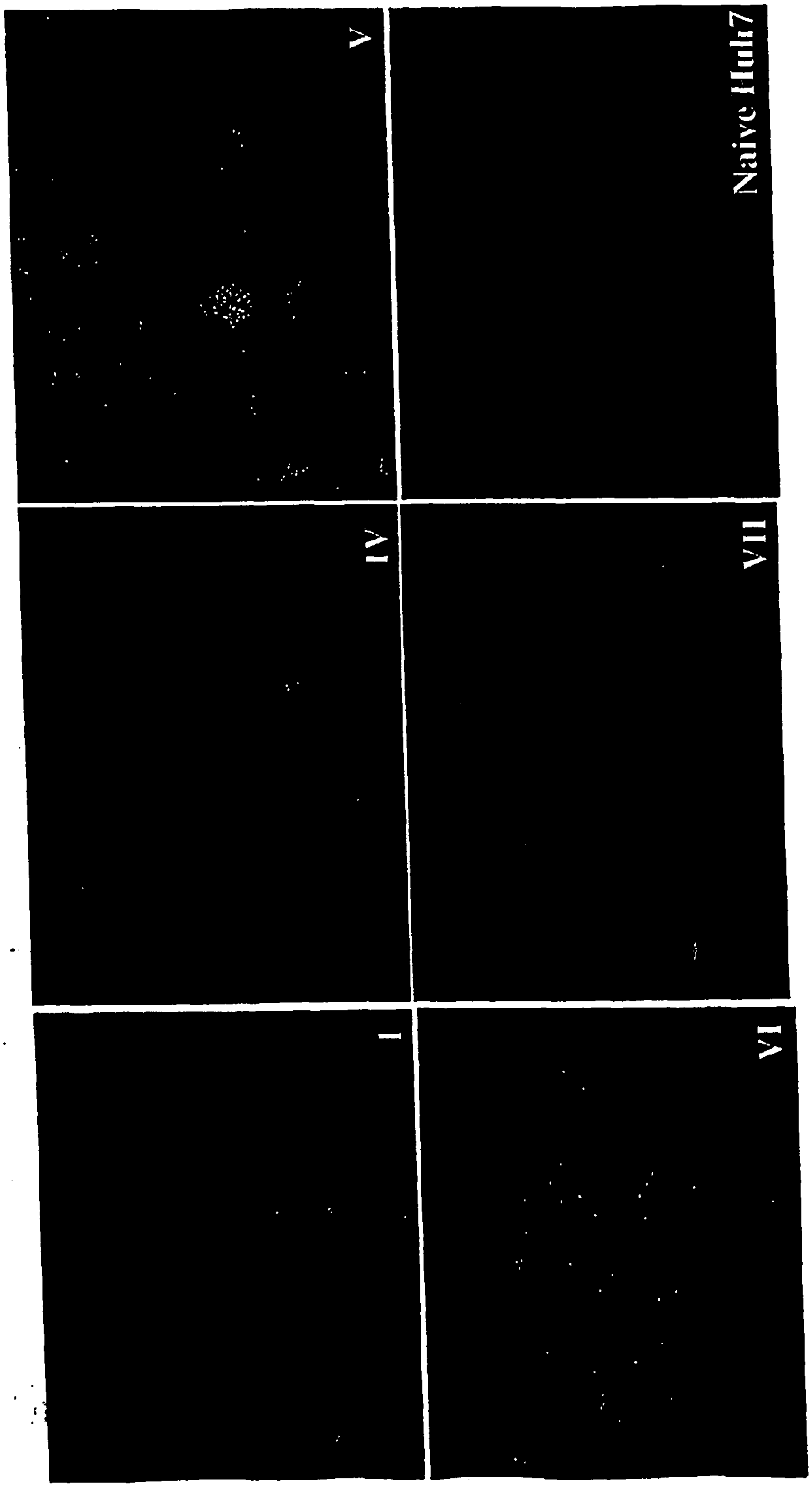


Figure 6

	aa 1163																			1182		1229
	Arg CGT	Arg AGG	Leu CTG	Ala GCC	Arg AGG	Gly GGA	Ser TCT	Pro CCC	Pro CCC	Ser TCC	Leu TTG	Ala GCC	Ser AGC	Ser TCA	Ser TCA	Ala GCT	Ser AGC	Gln CAG	Leu CTG	Ser TCT	//	Asp GAC
I	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	•	Tyr Δ47aa	••
II	•••	Gly GGG	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	//	•••
III	•••	•••	•••	•••	•••	•••	•••	•••	•••	Pro CCC	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	//	•••
IV	•••	•••	•••	•••	•••	•••	•••	•••	•••	Cys TGC	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	//	•••
V	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	Ser TCC	•••	•••	•••	•••	•••	•••	•••	•••	//	•••
VI/VII	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	•••	Ile ATC	•••	•••	•••	//	•••

Figure 7

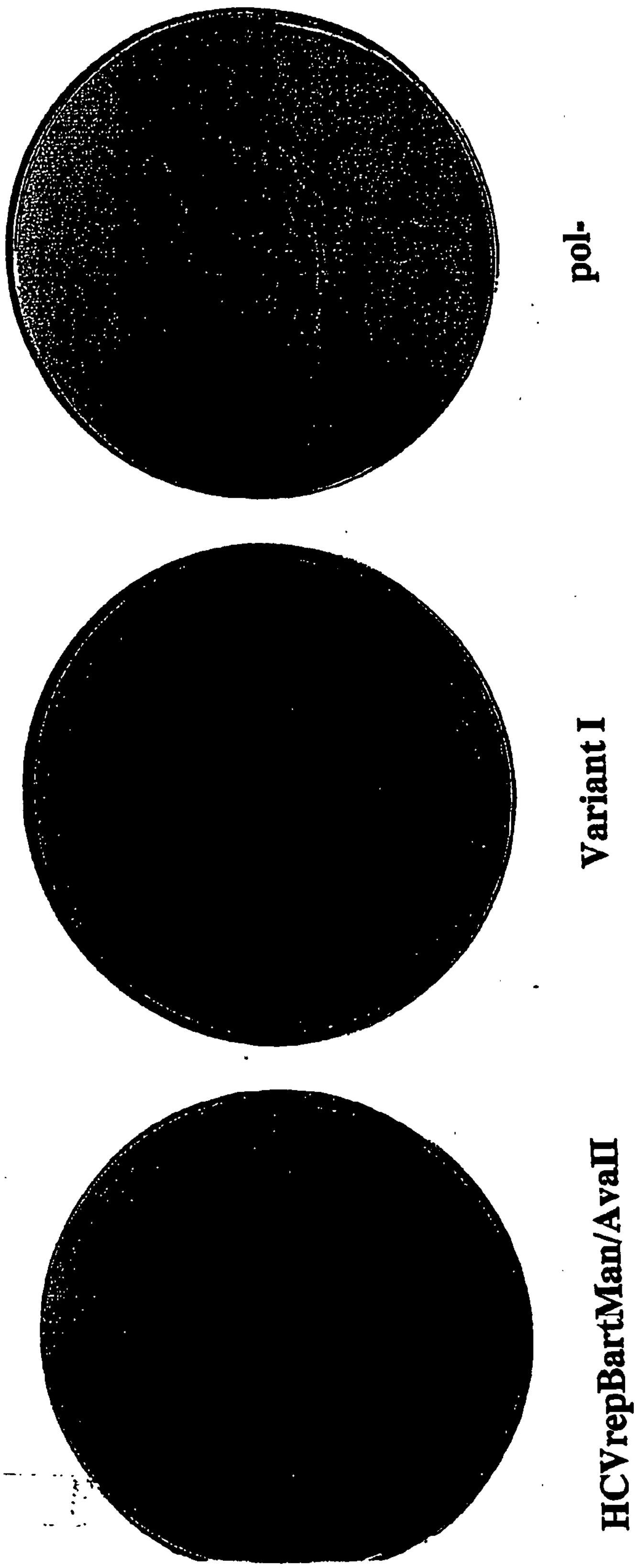


Figure 8

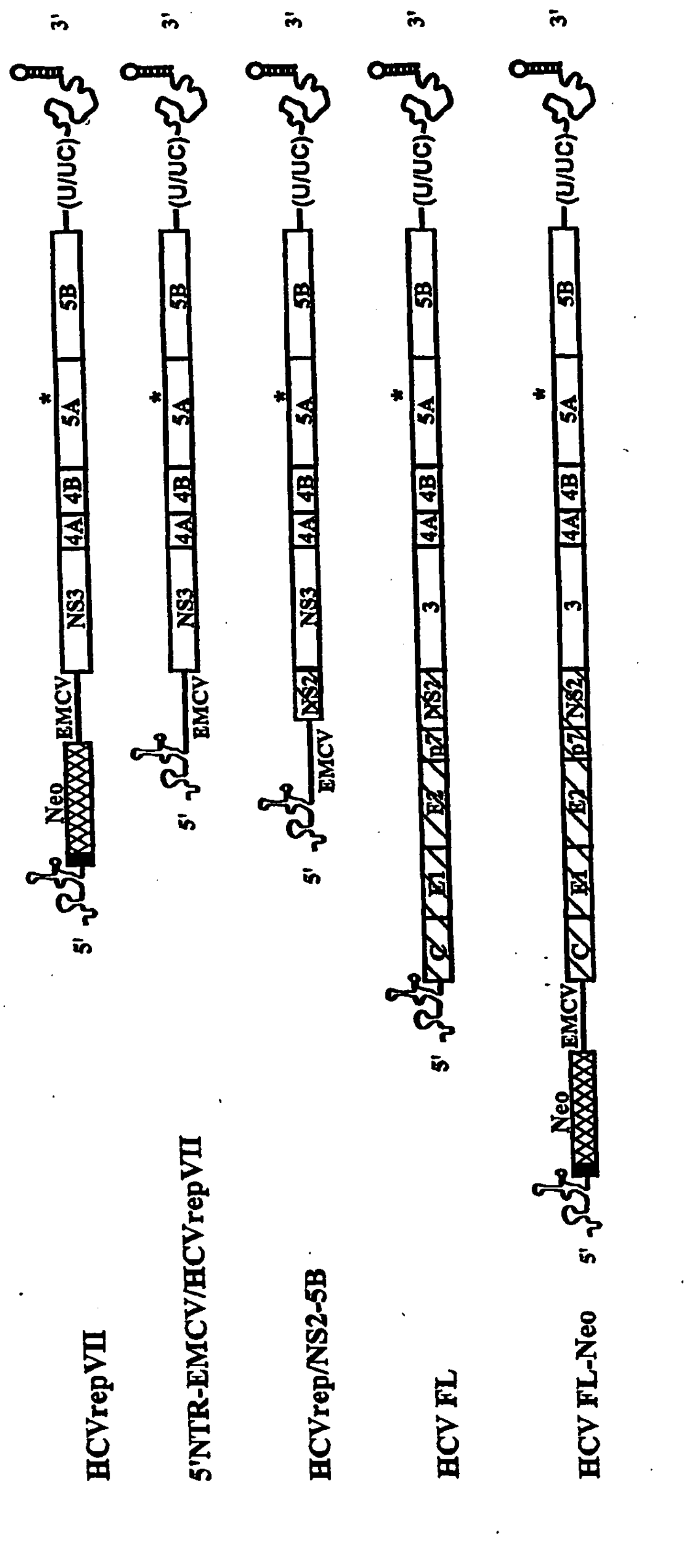

HCVrepVII
5'NTR-EMCV/HCVrepVII
HCVrep/NS2-5B
HCV FL
HCV FL-Neo
5'
3'
Neo
EMCV
NS2
NS3
4A
4B
5A
5B
C
E1
E2
p7
3
(U/UC)
*

Figure 9

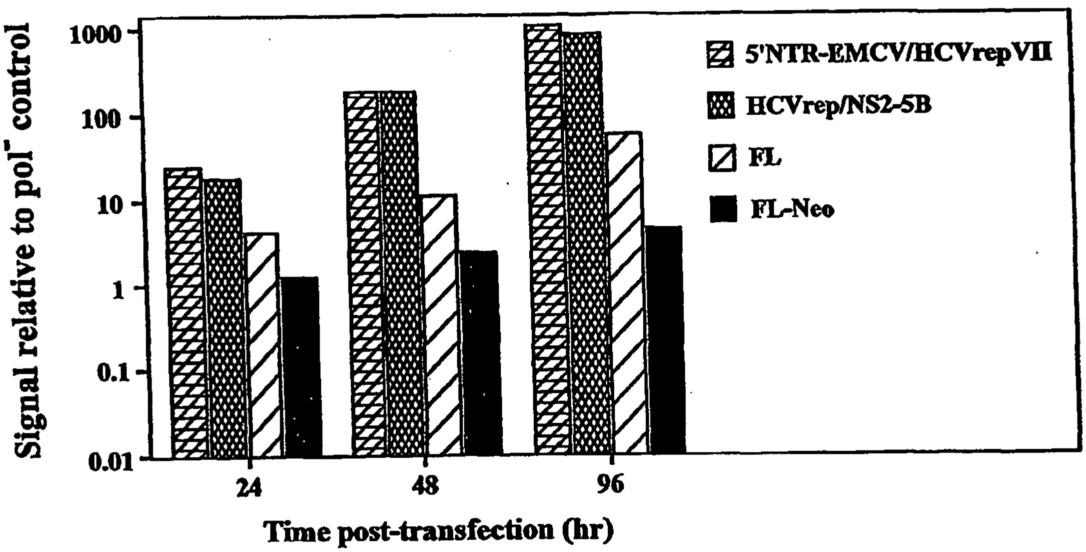

1000
100
10
1
0.1
0.01
Signal relative to pol- control
24
48
96
Time post-transfection (hr)
5'NTR-EMCV/HCVrepVII
HCVrep/NS2-5B
FL
FL-Neo

Figure 10

SEQUENCE LISTING

<110> Rice III, Charles
Blight, Keril

<120> HCV Variants

<130> 6029-7868

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<150> 09/576,989
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tgcagagagt gctgatactg gcctctctgc tgatcatgt 99

<210> 3
<211> 1985
<212> PRT
<213> Hepatitis C virus

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1 5 10 15

Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Arg Asn Gln Val Glu Gly
20 25 30

Glu Val Gln Val Val Ser Thr Ala Thr Gln Ser Phe Leu Ala Thr Cys
35 40 45

Val Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Ser Lys Thr
50 55 60

Leu Ala Gly Pro Lys Gly Pro Ile Thr Gln Met Tyr Thr Asn Val Asp
65 70 75 80

Gln Asp Leu Val Gly Trp Gln Ala Pro Pro Gly Ala Arg Ser Leu Thr
85 90 95

Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala
100 105 110

```
Asp Val Ile Pro Val Arg Arg Arg Gly Asp Ser Arg Gly Ser Leu Leu
        115                 120                 125

Ser Pro Arg Pro Val Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu
    130                 135                 140

Leu Cys Pro Ser Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys
145                 150                 155                 160

Thr Arg Gly Val Ala Lys Ala Val Asp Phe Val Pro Val Glu Ser Met
                165                 170                 175

Glu Thr Thr Met Arg Ser Pro Val Phe Thr Asp Asn Ser Ser Pro Pro
            180                 185                 190

Ala Val Pro Gln Thr Phe Gln Val Ala His Leu His Ala Pro Thr Gly
        195                 200                 205

Ser Gly Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly Tyr
    210                 215                 220

Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Leu Gly Phe Gly
225                 230                 235                 240

Ala Tyr Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr Gly
                245                 250                 255

Val Arg Thr Ile Thr Thr Gly Ala Pro Ile Thr Tyr Ser Thr Tyr Gly
            260                 265                 270

Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile
        275                 280                 285

Ile Cys Asp Glu Cys His Ser Thr Asp Ser Thr Thr Ile Leu Gly Ile
    290                 295                 300

Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Val Val
305                 310                 315                 320

Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn
                325                 330                 335

Ile Glu Glu Val Ala Leu Ser Ser Thr Gly Glu Ile Pro Phe Tyr Gly
            340                 345                 350

Lys Ala Ile Pro Ile Glu Thr Ile Lys Gly Gly Arg His Leu Ile Phe
        355                 360                 365

Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Ser Gly
    370                 375                 380

Leu Gly Leu Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val
385                 390                 395                 400

Ile Pro Thr Ser Gly Asp Val Ile Val Val Ala Thr Asp Ala Leu Met
                405                 410                 415

Thr Gly Phe Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys
            420                 425                 430

Val Thr Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu
        435                 440                 445

Thr Thr Thr Val Pro Gln Asp Ala Val Ser Arg Ser Gln Arg Arg Gly
    450                 455                 460
```

```
Arg Thr Gly Arg Gly Arg Met Gly Ile Tyr Arg Phe Val Thr Pro Gly
465                 470                 475                 480

Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr
                485                 490                 495

Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Ser Val
            500                 505                 510

Arg Leu Arg Ala Tyr Leu Asn Thr Pro Gly Leu Pro Val Cys Gln Asp
        515                 520                 525

His Leu Glu Phe Trp Glu Ser Val Phe Thr Gly Leu Thr His Ile Asp
    530                 535                 540

Ala His Phe Leu Ser Gln Thr Lys Gln Ala Gly Asp Asn Phe Pro Tyr
545                 550                 555                 560

Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro
                565                 570                 575

Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr
            580                 585                 590

Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn
        595                 600                 605

Glu Val Thr Thr Thr His Pro Ile Thr Lys Tyr Ile Met Ala Cys Met
    610                 615                 620

Ser Ala Asp Leu Glu Val Val Thr Ser Thr Trp Val Leu Val Gly Gly
625                 630                 635                 640

Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Thr Thr Gly Ser Val Val
                645                 650                 655

Ile Val Gly Arg Ile Ile Leu Ser Gly Lys Pro Ala Ile Ile Pro Asp
            660                 665                 670

Arg Glu Val Leu Tyr Arg Glu Phe Asp Glu Met Glu Glu Cys Ala Ser
        675                 680                 685

His Leu Pro Tyr Ile Glu Gln Gly Met Gln Leu Ala Glu Gln Phe Lys
    690                 695                 700

Gln Lys Ala Ile Gly Leu Leu Gln Thr Ala Thr Lys Gln Ala Glu Ala
705                 710                 715                 720

Ala Ala Pro Val Val Glu Ser Lys Trp Arg Thr Leu Glu Ala Phe Trp
                725                 730                 735

Ala Lys His Met Trp Asn Phe Ile Ser Gly Ile Gln Tyr Leu Ala Gly
            740                 745                 750

Leu Ser Thr Leu Pro Gly Asn Pro Ala Ile Ala Ser Leu Met Ala Phe
        755                 760                 765

Thr Ala Ser Ile Thr Ser Pro Leu Thr Thr Gln His Thr Leu Leu Phe
    770                 775                 780

Asn Ile Leu Gly Gly Trp Val Ala Ala Gln Leu Ala Pro Pro Ser Ala
785                 790                 795                 800

Ala Ser Ala Phe Val Gly Ala Gly Ile Ala Gly Ala Ala Val Gly Ser
                805                 810                 815
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Ile Gly Leu Gly Lys Val Leu Val Asp Ile Leu Ala Gly Tyr Gly Ala
820 825 830

Gly Val Ala Gly Ala Leu Val Ala Phe Lys Val Met Ser Gly Glu Met
835 840 845

Pro Ser Thr Glu Asp Leu Val Asn Leu Leu Pro Ala Ile Leu Ser Pro
850 855 860

Gly Ala Leu Val Val Gly Val Val Cys Ala Ala Ile Leu Arg Arg His
865 870 875 880

Val Gly Pro Gly Glu Gly Ala Val Gln Trp Met Asn Arg Leu Ile Ala
885 890 895

Phe Ala Ser Arg Gly Asn His Val Ser Pro Thr His Tyr Val Pro Glu
900 905 910

Ser Asp Ala Ala Ala Arg Val Thr Gln Ile Leu Ser Ser Leu Thr Ile
915 920 925

Thr Gln Leu Leu Lys Arg Leu His Gln Trp Ile Asn Glu Asp Cys Ser
930 935 940

Thr Pro Cys Ser Gly Ser Trp Leu Arg Asp Val Trp Asp Trp Ile Cys
945 950 955 960

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965 970 975

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980 985 990

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995 1000 1005

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1010 1015 1020

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1025 1030 1035 1040

Thr Thr Gly Pro Cys Thr Pro Ser Pro Ala Pro Asn Tyr Ser Arg Ala
1045 1050 1055

Leu Trp Arg Val Ala Ala Glu Glu Tyr Val Glu Val Thr Arg Val Gly
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Asp Phe His Tyr Val Thr Gly Met Thr Thr Asp Asn Val Lys Cys Pro
1075 1080 1085

Cys Gln Val Pro Ala Pro Glu Phe Phe Thr Glu Val Asp Gly Val Arg
1090 1095 1100

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1105 1110 1115 1120

Thr Phe Leu Val Gly Leu Asn Gln Tyr Leu Val Gly Ser Gln Leu Pro
1125 1130 1135

Cys Glu Pro Glu Pro Asp Val Ala Val Leu Thr Ser Met Leu Thr Asp
1140 1145 1150

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1155 1160 1165

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1170 1175 1180

Ser Leu Lys Ala Thr Cys Thr Thr Arg His Asp Ser Pro Asp Ala Asp
1185 1190 1195 1200

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Tyr Ala Pro Thr Leu Trp Ala Arg Met Ile Leu Met Thr His Phe Phe
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Ser Ile Leu Leu Ala Gln Glu Gln Leu Glu Lys Ala Leu Asp Cys Gln
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Ile Tyr Gly Ala Cys Tyr Ser Ile Glu Pro Leu Asp Leu Pro Gln Ile
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Ile Gln Arg Leu His Gly Leu Ser Ala Phe Ser Leu His Ser Tyr Ser
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Pro Gly Glu Ile Asn Arg Val Ala Ser Cys Leu Arg Lys Leu Gly Val
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Pro Pro Leu Arg Val Trp Arg His Arg Ala Arg Ser Val Arg Ala Arg
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<212> PRT
<213> Hepatitis C virus

<400> 14

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Gly Val Pro Phe Phe Ser Cys Gln Arg Gly Tyr Lys Gly Val Trp Arg
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Gly Asp Gly Ile Met Gln Thr Thr Cys Pro Cys Gly Ala Gln Ile Thr
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Gly His Val Lys Asn Gly Ser Met Arg Ile Val Gly Pro Arg Thr Cys
65                  70                  75                  80

Ser Asn Thr Trp His Gly Thr Phe Pro Ile Asn Ala Tyr Thr Thr Gly
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Pro Cys Thr Pro Ser Pro Ala Pro Asn Tyr Ser Arg Ala Leu Trp Arg
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Val Ala Ala Glu Glu Tyr Val Glu Val Thr Arg Val Gly Asp Phe His
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Tyr Val Thr Gly Met Thr Thr Asp Asn Val Lys Cys Pro Cys Gln Val
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Pro Ala Pro Glu Phe Phe Thr Glu Val Asp Gly Val Arg Leu His Arg
145                 150                 155                 160

Tyr Ala Pro Ala Cys Lys Pro Leu Leu Arg Glu Glu Val Thr Phe Leu
                165                 170                 175

Val Gly Leu Asn Gln Tyr Leu Val Gly Ser Gln Leu Pro Cys Glu Pro
            180                 185                 190

Glu Pro Asp Val Ala Val Leu Thr Ser Met Leu Thr Asp Pro Ser His
        195                 200                 205

Ile Thr Ala Glu Thr Ala Lys Arg Arg Leu Ala Arg Gly Ser Pro Pro
    210                 215                 220

Ser Leu Ala Ser Ser Ser Ala Ser Gln Leu Tyr Ser Phe Glu Pro Leu
225                 230                 235                 240

Gln Ala Glu Glu Asp Glu Arg Glu Val Ser Val Pro Ala Glu Ile Leu
                245                 250                 255

Arg Arg Ser Arg Lys Phe Pro Arg Ala Met Pro Ile Trp Ala Arg Pro
            260                 265                 270

Asp Tyr Asn Pro Pro Leu Leu Glu Ser Trp Lys Asp Pro Asp Tyr Val
        275                 280                 285

Pro Pro Val Val His Gly Cys Pro Leu Pro Pro Ala Lys Ala Pro Pro
    290                 295                 300

Ile Pro Pro Pro Arg Arg Lys Arg Thr Val Val Leu Ser Glu Ser Thr
305                 310                 315                 320

Val Ser Ser Ala Leu Ala Glu Leu Ala Thr Lys Thr Phe Gly Ser Ser
                325                 330                 335

Glu Ser Ser Ala Val Asp Ser Gly Thr Ala Thr Ala Ser Pro Asp Gln
            340                 345                 350

Pro Ser Asp Asp Gly Asp Ala Gly Ser Asp Val Glu Ser Tyr Ser Ser
        355                 360                 365

Met Pro Pro Leu Glu Gly Glu Pro Gly Asp Pro Asp Leu Ser Asp Gly
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Ser Trp Ser Thr Val Ser Glu Glu Ala Ser Glu Asp Val Val Cys Cys
385                 390                 395                 400


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<213> Hepatitis C virus

<400> 15
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Glu Val Gln Val Val Ser Thr Ala Thr Gln Ser Phe Leu Ala Thr Cys
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Val Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Ser Lys Thr
50 55 60

Leu Ala Gly Pro Lys Gly Pro Ile Thr Gln Met Tyr Thr Asn Val Asp
65 70 75 80

Gln Asp Leu Val Gly Trp Arg Ala Pro Pro Gly Ala Arg Ser Leu Thr
85 90 95

Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala
100 105 110

Asp Val Ile Pro Val Arg Arg Arg Gly Asp Ser Arg Gly Ser Leu Leu
115 120 125

Ser Pro Arg Pro Val Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu
130 135 140

Leu Cys Pro Ser Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys
145 150 155 160

Thr Arg Gly Val Ala Lys Ala Val Asp Phe Val Pro Val Glu Ser Met
165 170 175

Glu Thr Thr Met Arg Ser Pro Val Phe Thr Asp Asn Ser Ser Pro Pro
180 185 190

Ala Val Pro Gln Thr Phe Gln Val Ala His Leu His Ala Pro Thr Gly
195 200 205

Ser Gly Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly Tyr
210 215 220

Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Leu Gly Phe Gly
225 230 235 240

Ala Tyr Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr Gly
245 250 255

Val Arg Thr Ile Thr Thr Gly Ala Pro Ile Thr Tyr Ser Thr Tyr Gly
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Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile
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Ile Cys Asp Glu Cys His Ser Thr Asp Ser Thr Thr Ile Leu Gly Ile
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Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Val Val
305 310 315 320

Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn
325 330 335

Ile Glu Glu Val Ala Leu Ser Ser Thr Gly Glu Ile Pro Phe Tyr Gly
340 345 350

Lys Ala Ile Pro Ile Glu Thr Ile Lys Gly Gly Arg His Leu Ile Phe
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Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Ser Gly
370 375 380

Leu Gly Leu Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val
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Ile Pro Thr Ser Gly Asp Val Ile Val Val Ala Thr Asp Ala Leu Met
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Thr Gly Phe Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys
420 425 430

Val Thr Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu
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Arg Thr Gly Arg Gly Arg Met Gly Ile Tyr Arg Phe Val Thr Pro Gly
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Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr
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Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Ser Val
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Arg Leu Arg Ala Tyr Leu Asn Thr Pro Gly Leu Pro Val Cys Gln Asp
515 520 525

His Leu Glu Phe Trp Glu Ser Val Phe Thr Gly Leu Thr His Ile Asp
530 535 540

Ala His Phe Leu Ser Gln Thr Lys Gln Ala Gly Asp Asn Phe Pro Tyr
545 550 555 560

Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro
565 570 575

Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr
580 585 590

Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn
595 600 605

Glu Val Thr Thr Thr His Pro Ile Thr Lys Tyr Ile Met Ala Cys Met
610 615 620

Ser Ala Asp Leu Glu Val Val Thr Ser Thr Trp Val Leu Val Gly Gly
625 630 635 640

Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Thr Thr Gly Ser Val Val
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Ile Val Gly Arg Ile Ile Leu Ser Gly Lys Pro Ala Ile Ile Pro Asp
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Arg Glu Val Leu Tyr Arg Glu Phe Asp Glu Met Glu Glu Cys Ala Ser
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His Leu Pro Tyr Ile Glu Gln Gly Met Gln Leu Ala Glu Gln Phe Lys
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Gln Lys Ala Ile Gly Leu Leu Gln Thr Ala Thr Lys Gln Ala Glu Ala
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Ala Ala Pro Val Val Glu Ser Lys Trp Arg Thr Leu Glu Ala Phe Trp
725 730 735

Ala Lys His Met Trp Asn Phe Ile Ser Gly Ile Gln Tyr Leu Ala Gly
740 745 750

Leu Ser Thr Leu Pro Gly Asn Pro Ala Ile Ala Ser Leu Met Ala Phe
755 760 765

Thr Ala Ser Ile Thr Ser Pro Leu Thr Thr Gln His Thr Leu Leu Phe
770 775 780

Asn Ile Leu Gly Gly Trp Val Ala Ala Gln Leu Ala Pro Pro Ser Ala
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Ala Ser Ala Phe Val Gly Ala Gly Ile Ala Gly Ala Ala Val Gly Ser
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Ile Gly Leu Gly Lys Val Leu Val Asp Ile Leu Ala Gly Tyr Gly Ala
820 825 830

Gly Val Ala Gly Ala Leu Val Ala Phe Lys Val Met Ser Gly Glu Met
835 840 845

Pro Ser Thr Glu Asp Leu Val Asn Leu Leu Pro Ala Ile Leu Ser Pro
850 855 860

Gly Ala Leu Val Val Gly Val Val Cys Ala Ala Ile Leu Arg Arg His
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Val Gly Pro Gly Glu Gly Ala Val Gln Trp Met Asn Arg Leu Ile Ala
885 890 895

Phe Ala Ser Arg Gly Asn His Val Ser Pro Thr His Tyr Val Pro Glu
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Ser Asp Ala Ala Ala Arg Val Thr Gln Ile Leu Ser Ser Leu Thr Ile
915 920 925

Thr Gln Leu Leu Lys Arg Leu His Gln Trp Ile Asn Glu Asp Cys Ser
930 935 940

Thr Pro Cys Ser Gly Ser Trp Leu Arg Asp Val Trp Asp Trp Ile Cys
945 950 955 960

Thr Val Leu Thr Asp Phe Lys Thr Trp Leu Gln Ser Lys Leu Leu Pro
965 970 975

Arg Leu Pro Gly Val Pro Phe Phe Ser Cys Gln Arg Gly Tyr Lys Gly
980 985 990

Val Trp Arg Gly Asp Gly Ile Met Gln Thr Thr Cys Pro Cys Gly Ala
995 1000 1005

Gln Ile Thr Gly His Val Lys Asn Gly Ser Met Arg Ile Val Gly Pro
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Arg Thr Cys Ser Asn Thr Trp His Gly Thr Phe Pro Ile Asn Ala Tyr
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Thr Thr Gly Pro Cys Thr Pro Ser Pro Ala Pro Asn Tyr Ser Arg Ala
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Leu Trp Arg Val Ala Ala Glu Glu Tyr Val Glu Val Thr Arg Val Gly
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Ser Leu Lys Ala Thr Cys Thr Thr Arg His Asp Ser Pro Asp Ala Asp
1185 1190 1195 1200

Leu Ile Glu Ala Asn Leu Leu Trp Arg Gln Glu Met Gly Gly Asn Ile
1205 1210 1215

Thr Arg Val Glu Ser Glu Asn Lys Val Val Ile Leu Asp Ser Phe Glu
1220 1225 1230

Pro Leu Gln Ala Glu Glu Asp Glu Arg Glu Val Ser Val Pro Ala Glu
1235 1240 1245

Ile Leu Arg Arg Ser Arg Lys Phe Pro Arg Ala Met Pro Ile Trp Ala
1250 1255 1260

Arg Pro Asp Tyr Asn Pro Pro Leu Leu Glu Ser Trp Lys Asp Pro Asp
1265 1270 1275 1280

Tyr Val Pro Pro Val Val His Gly Cys Pro Leu Pro Pro Ala Lys Ala
1285 1290 1295

Pro Pro Ile Pro Pro Pro Arg Arg Lys Arg Thr Val Val Leu Ser Glu
1300 1305 1310

Ser Thr Val Ser Ser Ala Leu Ala Glu Leu Ala Thr Lys Thr Phe Gly
1315 1320 1325

Ser Ser Glu Ser Ser Ala Val Asp Ser Gly Thr Ala Thr Ala Ser Pro
1330 1335 1340

Asp Gln Pro Ser Asp Asp Gly Asp Ala Gly Ser Asp Val Glu Ser Tyr
1345 1350 1355 1360

Ser Ser Met Pro Pro Leu Glu Gly Glu Pro Gly Asp Pro Asp Leu Ser
1365 1370 1375

Asp Gly Ser Trp Ser Thr Val Ser Glu Glu Ala Ser Glu Asp Val Val
1380 1385 1390

Cys Cys Ser Met Ser Tyr Thr Trp Thr Gly Ala Leu Ile Thr Pro Cys
1395 1400 1405

Ala Ala Glu Glu Thr Lys Leu Pro Ile Asn Ala Leu Ser Asn Ser Leu
1410 1415 1420

Leu Arg His His Asn Leu Val Tyr Ala Thr Thr Ser Arg Ser Ala Ser
1425 1430 1435 1440

Leu Arg Gln Lys Lys Val Thr Phe Asp Arg Leu Gln Val Leu Asp Asp
1445 1450 1455

His Tyr Arg Asp Val Leu Lys Glu Met Lys Ala Lys Ala Ser Thr Val
1460 1465 1470

Lys Ala Lys Leu Leu Ser Val Glu Glu Ala Cys Lys Leu Thr Pro Pro
1475 1480 1485

His Ser Ala Arg Ser Lys Phe Gly Tyr Gly Ala Lys Asp Val Arg Asn
1490 1495 1500

Leu Ser Ser Lys Ala Val Asn His Ile Arg Ser Val Trp Lys Asp Leu
1505 1510 1515 1520

Leu Glu Asp Thr Glu Thr Pro Ile Asp Thr Thr Ile Met Ala Lys Asn
1525 1530 1535

Glu Val Phe Cys Val Gln Pro Glu Lys Gly Gly Arg Lys Pro Ala Arg
1540 1545 1550

Leu Ile Val Phe Pro Asp Leu Gly Val Arg Val Cys Glu Lys Met Ala
1555 1560 1565

Leu Tyr Asp Val Val Ser Thr Leu Pro Gln Ala Val Met Gly Ser Ser
1570 1575 1580

Tyr Gly Phe Gln Tyr Ser Pro Gly Gln Arg Val Glu Phe Leu Val Asn
1585 1590 1595 1600

Ala Trp Lys Ala Lys Lys Cys Pro Met Gly Phe Ala Tyr Asp Thr Arg
1605 1610 1615

Cys Phe Asp Ser Thr Val Thr Glu Asn Asp Ile Arg Val Glu Glu Ser
1620 1625 1630

Ile Tyr Gln Cys Cys Asp Leu Ala Pro Glu Ala Arg Gln Ala Ile Arg
1635 1640 1645

Ser Leu Thr Glu Arg Leu Tyr Ile Gly Gly Pro Leu Thr Asn Ser Lys
1650 1655 1660

Gly Gln Asn Cys Gly Tyr Arg Arg Cys Arg Ala Ser Gly Val Leu Thr
1665 1670 1675 1680

Thr Ser Cys Gly Asn Thr Leu Thr Cys Tyr Leu Lys Ala Ala Ala Ala
1685 1690 1695

Cys Arg Ala Ala Lys Leu Gln Asp Cys Thr Met Leu Val Cys Gly Asp
1700 1705 1710

Asp Leu Val Val Ile Cys Glu Ser Ala Gly Thr Gln Glu Asp Glu Ala
1715 1720 1725

Ser Leu Arg Ala Phe Thr Glu Ala Met Thr Arg Tyr Ser Ala Pro Pro
1730 1735 1740

Gly Asp Pro Pro Lys Pro Glu Tyr Asp Leu Glu Leu Ile Thr Ser Cys
1745 1750 1755 1760

Ser Ser Asn Val Ser Val Ala His Asp Ala Ser Gly Lys Arg Val Tyr
1765 1770 1775

Tyr Leu Thr Arg Asp Pro Thr Thr Pro Leu Ala Arg Ala Ala Trp Glu
1780 1785 1790

Thr Ala Arg His Thr Pro Val Asn Ser Trp Leu Gly Asn Ile Ile Met
1795 1800 1805

Tyr Ala Pro Thr Leu Trp Ala Arg Met Ile Leu Met Thr His Phe Phe
1810 1815 1820

Ser Ile Leu Leu Ala Gln Glu Gln Leu Glu Lys Ala Leu Asp Cys Gln
1825 1830 1835 1840

Ile Tyr Gly Ala Cys Tyr Ser Ile Glu Pro Leu Asp Leu Pro Gln Ile
1845 1850 1855

Ile Gln Arg Leu His Gly Leu Ser Ala Phe Ser Leu His Ser Tyr Ser
1860 1865 1870

Pro Gly Glu Ile Asn Arg Val Ala Ser Cys Leu Arg Lys Leu Gly Val
1875 1880 1885

Pro Pro Leu Arg Val Trp Arg His Arg Ala Arg Ser Val Arg Ala Arg
1890 1895 1900

Leu Leu Ser Gln Gly Gly Arg Ala Ala Thr Cys Gly Lys Tyr Leu Phe
1905 1910 1915 1920

Asn Trp Ala Val Arg Thr Lys Leu Lys Leu Thr Pro Ile Pro Ala Ala
1925 1930 1935

Ser Gln Leu Asp Leu Ser Ser Trp Phe Val Ala Gly Tyr Ser Gly Gly
1940 1945 1950

Asp Ile Tyr His Ser Leu Ser Arg Ala Arg Pro Arg Trp Phe Met Trp
1955 1960 1965

Cys Leu Leu Leu Leu Ser Val Gly Val Gly Ile Tyr Leu Leu Pro Asn
1970 1975 1980

Arg
1985

<210> 16
<211> 447
<212> PRT
<213> Hepatitis C virus

<400> 16
Ser Gly Ser Trp Leu Arg Asp Val Trp Asp Trp Ile Cys Thr Val Leu
1 5 10 15

Thr Asp Phe Lys Thr Trp Leu Gln Ser Lys Leu Leu Pro Arg Leu Pro
20 25 30

Gly Val Pro Phe Phe Ser Cys Gln Arg Gly Tyr Lys Gly Val Trp Arg
35 40 45

Gly Asp Gly Ile Met Gln Thr Thr Cys Pro Cys Gly Ala Gln Ile Thr
50 55 60

Gly His Val Lys Asn Gly Ser Met Arg Ile Val Gly Pro Arg Thr Cys
65 70 75 80

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Ser Asn Thr Trp His Gly Thr Phe Pro Ile Asn Ala Tyr Thr Thr Gly
                85                  90                  95

Pro Cys Thr Pro Ser Pro Ala Pro Asn Tyr Ser Arg Ala Leu Trp Arg
            100                 105                 110

Val Ala Ala Glu Glu Tyr Val Glu Val Thr Arg Val Gly Asp Phe His
        115                 120                 125

Tyr Val Thr Gly Met Thr Thr Asp Asn Val Lys Cys Pro Cys Gln Val
    130                 135                 140

Pro Ala Pro Glu Phe Phe Thr Glu Val Asp Gly Val Arg Leu His Arg
145                 150                 155                 160

Tyr Ala Pro Ala Cys Lys Pro Leu Leu Arg Glu Glu Val Thr Phe Leu
                165                 170                 175

Val Gly Leu Asn Gln Tyr Leu Val Gly Ser Gln Leu Pro Cys Glu Pro
            180                 185                 190

Glu Pro Asp Val Ala Val Leu Thr Ser Met Leu Thr Asp Pro Ser His
        195                 200                 205

Ile Thr Ala Glu Thr Ala Lys Arg Arg Leu Ala Arg Gly Ser Pro Pro
    210                 215                 220

Ser Leu Ala Ser Ser Ser Ala Ile Gln Leu Ser Ala Pro Ser Leu Lys
225                 230                 235                 240

Ala Thr Cys Thr Thr Arg His Asp Ser Pro Asp Ala Asp Leu Ile Glu
                245                 250                 255

Ala Asn Leu Leu Trp Arg Gln Glu Met Gly Gly Asn Ile Thr Arg Val
            260                 265                 270

Glu Ser Glu Asn Lys Val Val Ile Leu Asp Ser Phe Glu Pro Leu Gln
        275                 280                 285

Ala Glu Glu Asp Glu Arg Glu Val Ser Val Pro Ala Glu Ile Leu Arg
    290                 295                 300

Arg Ser Arg Lys Phe Pro Arg Ala Met Pro Ile Trp Ala Arg Pro Asp
305                 310                 315                 320

Tyr Asn Pro Pro Leu Leu Glu Ser Trp Lys Asp Pro Asp Tyr Val Pro
                325                 330                 335

Pro Val Val His Gly Cys Pro Leu Pro Pro Ala Lys Ala Pro Pro Ile
            340                 345                 350

Pro Pro Pro Arg Arg Lys Arg Thr Val Val Leu Ser Glu Ser Thr Val
        355                 360                 365

Ser Ser Ala Leu Ala Glu Leu Ala Thr Lys Thr Phe Gly Ser Ser Glu
    370                 375                 380

Ser Ser Ala Val Asp Ser Gly Thr Ala Thr Ala Ser Pro Asp Gln Pro
385                 390                 395                 400

Ser Asp Asp Gly Asp Ala Gly Ser Asp Val Glu Ser Tyr Ser Ser Met
                405                 410                 415

Pro Pro Leu Glu Gly Glu Pro Gly Asp Pro Asp Leu Ser Asp Gly Ser
            420                 425                 430
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Trp Ser Thr Val Ser Glu Glu Ala Ser Glu Asp Val Val Cys Cys
435 440 445

<210> 17
<211> 1985
<212> PRT
<213> Hepatitis C virus

<400> 17
Met Ala Pro Ile Thr Ala Tyr Ser Gln Gln Thr Arg Gly Leu Leu Gly
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Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Arg Asn Gln Val Glu Gly
20 25 30

Glu Val Gln Val Val Ser Thr Ala Thr Gln Ser Phe Leu Ala Thr Cys
35 40 45

Val Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Ser Lys Thr
50 55 60

Leu Ala Gly Pro Lys Gly Pro Ile Thr Gln Met Tyr Thr Asn Val Asp
65 70 75 80

Gln Asp Leu Val Gly Trp Gln Ala Pro Pro Gly Ala Arg Ser Leu Thr
85 90 95

Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala
100 105 110

Asp Val Ile Pro Val Arg Arg Arg Gly Asp Ser Arg Gly Ser Leu Leu
115 120 125

Ser Pro Arg Pro Val Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu
130 135 140

Leu Cys Pro Ser Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys
145 150 155 160

Thr Arg Gly Val Ala Lys Ala Val Asp Phe Val Pro Val Glu Ser Met
165 170 175

Glu Thr Thr Met Arg Ser Pro Val Phe Thr Asp Asn Ser Ser Pro Pro
180 185 190

Ala Val Pro Gln Thr Phe Gln Val Ala His Leu His Ala Pro Thr Gly
195 200 205

Ser Gly Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly Tyr
210 215 220

Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Leu Gly Phe Gly
225 230 235 240

Ala Tyr Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr Gly
245 250 255

Val Arg Thr Ile Thr Thr Gly Ala Pro Ile Thr Tyr Ser Thr Tyr Gly
260 265 270

Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile
275 280 285

Ile Cys Asp Glu Cys His Ser Thr Asp Ser Thr Thr Ile Leu Gly Ile
290 295 300

Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Val Val
305 310 315 320

Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn
325 330 335

Ile Glu Glu Val Ala Leu Ser Ser Thr Gly Glu Ile Pro Phe Tyr Gly
340 345 350

Lys Ala Ile Pro Ile Glu Thr Ile Lys Gly Gly Arg His Leu Ile Phe
355 360 365

Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Ser Gly
370 375 380

Leu Gly Leu Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val
385 390 395 400

Ile Pro Thr Ser Gly Asp Val Ile Val Val Ala Thr Asp Ala Leu Met
405 410 415

Thr Gly Phe Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys
420 425 430

Val Thr Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu
435 440 445

Thr Thr Thr Val Pro Gln Asp Ala Val Ser Arg Ser Gln Arg Arg Gly
450 455 460

Arg Thr Gly Arg Gly Arg Met Gly Ile Tyr Arg Phe Val Thr Pro Gly
465 470 475 480

Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr
485 490 495

Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Ser Val
500 505 510

Arg Leu Arg Ala Tyr Leu Asn Thr Pro Gly Leu Pro Val Cys Gln Asp
515 520 525

His Leu Glu Phe Trp Glu Ser Val Phe Thr Gly Leu Thr His Ile Asp
530 535 540

Ala His Phe Leu Ser Gln Thr Lys Gln Ala Gly Asp Asn Phe Pro Tyr
545 550 555 560

Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro
565 570 575

Pro Ser Trp Asp Gln Met Trp Glu Cys Leu Ile Arg Leu Lys Pro Thr
580 585 590

Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn
595 600 605

Glu Val Thr Thr Thr His Pro Ile Thr Lys Tyr Ile Met Ala Cys Met
610 615 620

Ser Ala Asp Leu Glu Val Val Thr Ser Thr Trp Val Leu Val Gly Gly
625 630 635 640

Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Thr Thr Gly Ser Val Val
645 650 655

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Ile Val Gly Arg Ile Ile Leu Ser Gly Lys Pro Ala Ile Ile Pro Asp
            660                 665                 670

Arg Glu Val Leu Tyr Arg Glu Phe Asp Glu Met Glu Glu Cys Ala Ser
        675                 680                 685

His Leu Pro Tyr Ile Glu Gln Gly Met Gln Leu Ala Glu Gln Phe Lys
    690                 695                 700

Gln Lys Ala Ile Gly Leu Leu Gln Thr Ala Thr Lys Gln Ala Glu Ala
705                 710                 715                 720

Ala Ala Pro Val Val Glu Ser Lys Trp Arg Thr Leu Glu Ala Phe Trp
                725                 730                 735

Ala Lys His Met Trp Asn Phe Ile Ser Gly Ile Gln Tyr Leu Ala Gly
            740                 745                 750

Leu Ser Thr Leu Pro Gly Asn Pro Ala Ile Ala Ser Leu Met Ala Phe
        755                 760                 765

Thr Ala Ser Ile Thr Ser Pro Leu Thr Thr Gln His Thr Leu Leu Phe
    770                 775                 780

Asn Ile Leu Gly Gly Trp Val Ala Ala Gln Leu Ala Pro Pro Ser Ala
785                 790                 795                 800

Ala Ser Ala Phe Val Gly Ala Gly Ile Ala Gly Ala Ala Val Gly Ser
                805                 810                 815

Ile Gly Leu Gly Lys Val Leu Val Asp Ile Leu Ala Gly Tyr Gly Ala
            820                 825                 830

Gly Val Ala Gly Ala Leu Val Ala Phe Lys Val Met Ser Gly Glu Met
        835                 840                 845

Pro Ser Thr Glu Asp Leu Val Asn Leu Leu Pro Ala Ile Leu Ser Pro
    850                 855                 860

Gly Ala Leu Val Val Gly Val Val Cys Ala Ala Ile Leu Arg Arg His
865                 870                 875                 880

Val Gly Pro Gly Glu Gly Ala Val Gln Trp Met Asn Arg Leu Ile Ala
                885                 890                 895

Phe Ala Ser Arg Gly Asn His Val Ser Pro Thr His Tyr Val Pro Glu
            900                 905                 910

Ser Asp Ala Ala Ala Arg Val Thr Gln Ile Leu Ser Gly Leu Thr Ile
        915                 920                 925

Thr Gln Leu Leu Lys Arg Leu His Gln Trp Ile Asn Glu Asp Cys Ser
    930                 935                 940

Thr Pro Cys Ser Gly Ser Trp Leu Arg Asp Val Trp Asp Trp Ile Cys
945                 950                 955                 960

Thr Val Leu Thr Asp Phe Lys Thr Trp Leu Gln Ser Lys Leu Leu Pro
                965                 970                 975

Arg Leu Pro Gly Val Pro Phe Phe Ser Cys Gln Arg Gly Tyr Lys Gly
            980                 985                 990

Val Trp Arg Gly Asp Gly Ile Met Gln Thr Thr Cys Pro Cys Gly Ala
        995                 1000                1005
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Gln Ile Thr Gly His Val Lys Asn Gly Ser Met Arg Ile Val Gly Pro
1010 1015 1020

Arg Thr Cys Ser Asn Thr Trp His Gly Thr Phe Pro Ile Asn Ala Tyr
1025 1030 1035 1040

Thr Thr Gly Pro Cys Thr Pro Ser Pro Ala Pro Asn Tyr Ser Arg Ala
1045 1050 1055

Leu Trp Arg Val Ala Ala Glu Glu Tyr Val Glu Val Thr Arg Val Gly
1060 1065 1070

Asp Phe His Tyr Val Thr Gly Met Thr Thr Asp Asn Val Lys Cys Pro
1075 1080 1085

Cys Gln Val Pro Ala Pro Glu Phe Phe Thr Glu Val Asp Gly Val Arg
1090 1095 1100

Leu His Arg Tyr Ala Pro Ala Cys Lys Pro Leu Leu Arg Glu Glu Val
1105 1110 1115 1120

Thr Phe Leu Val Gly Leu Asn Gln Tyr Leu Val Gly Ser Gln Leu Pro
1125 1130 1135

Cys Glu Pro Glu Pro Asp Val Ala Val Leu Thr Ser Met Leu Thr Asp
1140 1145 1150

Pro Ser His Ile Thr Ala Glu Thr Ala Lys Arg Gly Leu Ala Arg Gly
1155 1160 1165

Ser Pro Pro Ser Leu Ala Ser Ser Ser Ala Ser Gln Leu Ser Ala Pro
1170 1175 1180

Ser Leu Lys Ala Thr Cys Thr Thr Arg His Asp Ser Pro Asp Ala Asp
1185 1190 1195 1200

Leu Ile Glu Ala Asn Leu Leu Trp Arg Gln Glu Met Gly Gly Asn Ile
1205 1210 1215

Thr Arg Val Glu Ser Glu Asn Lys Val Val Ile Leu Asp Ser Phe Glu
1220 1225 1230

Pro Leu Gln Ala Glu Glu Asp Glu Arg Glu Val Ser Val Pro Ala Glu
1235 1240 1245

Ile Leu Arg Arg Ser Arg Lys Phe Pro Arg Ala Met Pro Ile Trp Ala
1250 1255 1260

Arg Pro Asp Tyr Asn Pro Pro Leu Leu Glu Ser Trp Lys Asp Pro Asp
1265 1270 1275 1280

Tyr Val Pro Pro Val Val His Gly Cys Pro Leu Pro Pro Ala Lys Ala
1285 1290 1295

Pro Pro Ile Pro Pro Pro Arg Arg Lys Arg Thr Val Val Leu Ser Glu
1300 1305 1310

Ser Thr Val Ser Ser Ala Leu Ala Glu Leu Ala Thr Lys Thr Phe Gly
1315 1320 1325

Ser Ser Glu Ser Ser Ala Val Asp Ser Gly Thr Ala Thr Ala Ser Pro
1330 1335 1340

Asp Gln Pro Ser Asp Asp Gly Asp Ala Gly Ser Asp Val Glu Ser Tyr
1345 1350 1355 1360

Ser Ser Met Pro Pro Leu Glu Gly Glu Pro Gly Asp Pro Asp Leu Ser
1365 1370 1375

Asp Gly Ser Trp Ser Thr Val Ser Glu Glu Ala Ser Glu Asp Val Val
1380 1385 1390

Cys Cys Ser Met Ser Tyr Thr Trp Thr Gly Ala Leu Ile Thr Pro Cys
1395 1400 1405

Ala Ala Glu Glu Thr Lys Leu Pro Ile Asn Ala Leu Ser Asn Ser Leu
1410 1415 1420

Leu Arg His His Asn Leu Val Tyr Ala Thr Thr Ser Arg Ser Ala Ser
1425 1430 1435 1440

Leu Arg Gln Lys Lys Val Thr Phe Asp Arg Leu Gln Val Leu Asp Asp
1445 1450 1455

His Tyr Arg Asp Val Leu Lys Glu Met Lys Ala Lys Ala Ser Thr Val
1460 1465 1470

Lys Ala Lys Leu Leu Ser Val Glu Glu Ala Cys Lys Leu Thr Pro Pro
1475 1480 1485

His Ser Ala Arg Ser Lys Phe Gly Tyr Gly Ala Lys Asp Val Arg Asn
1490 1495 1500

Leu Ser Ser Lys Ala Val Asn His Ile Arg Ser Val Trp Lys Asp Leu
1505 1510 1515 1520

Leu Glu Asp Thr Glu Thr Pro Ile Asp Thr Thr Ile Met Ala Lys Asn
1525 1530 1535

Glu Val Phe Cys Val Gln Pro Glu Lys Gly Gly Arg Lys Pro Ala Arg
1540 1545 1550

Leu Ile Val Phe Pro Asp Leu Gly Val Arg Val Cys Glu Lys Met Ala
1555 1560 1565

Leu Tyr Asp Val Val Ser Thr Leu Pro Gln Ala Val Met Gly Ser Ser
1570 1575 1580

Tyr Gly Phe Gln Tyr Ser Pro Gly Gln Arg Val Glu Phe Leu Val Asn
1585 1590 1595 1600

Ala Trp Lys Ala Lys Lys Cys Pro Met Gly Phe Ala Tyr Asp Thr Arg
1605 1610 1615

Cys Phe Asp Ser Thr Val Thr Glu Asn Asp Ile Arg Val Glu Glu Ser
1620 1625 1630

Ile Tyr Gln Cys Cys Asp Leu Ala Pro Glu Ala Arg Gln Ala Ile Arg
1635 1640 1645

Ser Leu Thr Glu Arg Leu Tyr Ile Gly Gly Pro Leu Thr Asn Ser Lys
1650 1655 1660

Gly Gln Asn Cys Gly Tyr Arg Arg Cys Arg Ala Ser Gly Val Leu Thr
1665 1670 1675 1680

Thr Ser Cys Gly Asn Thr Leu Thr Cys Tyr Leu Lys Ala Ala Ala Ala
1685 1690 1695

Cys Arg Ala Ala Lys Leu Gln Asp Cys Thr Met Leu Val Cys Gly Asp
1700 1705 1710

Asp Leu Val Val Ile Cys Glu Ser Ala Gly Thr Gln Glu Asp Glu Ala
1715 1720 1725

Ser Leu Arg Ala Phe Thr Glu Ala Met Thr Arg Tyr Ser Ala Pro Pro
1730 1735 1740

Gly Asp Pro Pro Lys Pro Glu Tyr Asp Leu Glu Leu Ile Thr Ser Cys
1745 1750 1755 1760

Ser Ser Asn Val Ser Val Ala His Asp Ala Ser Gly Lys Arg Val Tyr
1765 1770 1775

Tyr Leu Thr Arg Asp Pro Thr Thr Pro Leu Ala Arg Ala Ala Trp Glu
1780 1785 1790

Thr Ala Arg His Thr Pro Val Asn Ser Trp Leu Gly Asn Ile Ile Met
1795 1800 1805

Tyr Ala Pro Thr Leu Trp Ala Arg Met Ile Leu Met Thr His Phe Phe
1810 1815 1820

Ser Ile Leu Leu Ala Gln Glu Gln Leu Glu Lys Ala Leu Asp Cys Gln
1825 1830 1835 1840

Ile Tyr Gly Ala Cys Tyr Ser Ile Glu Pro Leu Asp Leu Pro Gln Ile
1845 1850 1855

Ile Gln Arg Leu His Gly Leu Ser Ala Phe Ser Leu His Ser Tyr Ser
1860 1865 1870

Pro Gly Glu Ile Asn Arg Val Ala Ser Cys Leu Arg Lys Leu Gly Val
1875 1880 1885

Pro Pro Leu Arg Val Trp Arg His Arg Ala Arg Ser Val Arg Ala Arg
1890 1895 1900

Leu Leu Ser Gln Gly Gly Arg Ala Ala Thr Cys Gly Lys Tyr Leu Phe
1905 1910 1915 1920

Asn Trp Ala Val Arg Thr Lys Leu Lys Leu Thr Pro Ile Pro Ala Ala
1925 1930 1935

Ser Gln Leu Asp Leu Ser Ser Trp Phe Val Ala Gly Tyr Ser Gly Gly
1940 1945 1950

Asp Ile Tyr His Ser Leu Ser Arg Ala Arg Pro Arg Trp Phe Met Trp
1955 1960 1965

Cys Leu Leu Leu Leu Ser Val Gly Val Gly Ile Tyr Leu Leu Pro Asn
1970 1975 1980

Arg
1985

<210> 18
<211> 447
<212> PRT
<213> Hepatitis C virus

<400> 18
Ser Gly Ser Trp Leu Arg Asp Val Trp Asp Trp Ile Cys Thr Val Leu
1 5 10 15

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Thr Asp Phe Lys Thr Trp Leu Gln Ser Lys Leu Leu Pro Arg Leu Pro
            20                  25                  30

Gly Val Pro Phe Phe Ser Cys Gln Arg Gly Tyr Lys Gly Val Trp Arg
        35                  40                  45

Gly Asp Gly Ile Met Gln Thr Thr Cys Pro Cys Gly Ala Gln Ile Thr
    50                  55                  60

Gly His Val Lys Asn Gly Ser Met Arg Ile Val Gly Pro Arg Thr Cys
65                  70                  75                  80

Ser Asn Thr Trp His Gly Thr Phe Pro Ile Asn Ala Tyr Thr Thr Gly
                85                  90                  95

Pro Cys Thr Pro Ser Pro Ala Pro Asn Tyr Ser Arg Ala Leu Trp Arg
            100                 105                 110

Val Ala Ala Glu Glu Tyr Val Glu Val Thr Arg Val Gly Asp Phe His
        115                 120                 125

Tyr Val Thr Gly Met Thr Thr Asp Asn Val Lys Cys Pro Cys Gln Val
    130                 135                 140

Pro Ala Pro Glu Phe Phe Thr Glu Val Asp Gly Val Arg Leu His Arg
145                 150                 155                 160

Tyr Ala Pro Ala Cys Lys Pro Leu Leu Arg Glu Glu Val Thr Phe Leu
                165                 170                 175

Val Gly Leu Asn Gln Tyr Leu Val Gly Ser Gln Leu Pro Cys Glu Pro
            180                 185                 190

Glu Pro Asp Val Ala Val Leu Thr Ser Met Leu Thr Asp Pro Ser His
        195                 200                 205

Ile Thr Ala Glu Thr Ala Lys Arg Gly Leu Ala Arg Gly Ser Pro Pro
    210                 215                 220

Ser Leu Ala Ser Ser Ser Ala Ser Gln Leu Ser Ala Pro Ser Leu Lys
225                 230                 235                 240

Ala Thr Cys Thr Thr Arg His Asp Ser Pro Asp Ala Asp Leu Ile Glu
                245                 250                 255

Ala Asn Leu Leu Trp Arg Gln Glu Met Gly Gly Asn Ile Thr Arg Val
            260                 265                 270

Glu Ser Glu Asn Lys Val Val Ile Leu Asp Ser Phe Glu Pro Leu Gln
        275                 280                 285

Ala Glu Glu Asp Glu Arg Glu Val Ser Val Pro Ala Glu Ile Leu Arg
    290                 295                 300

Arg Ser Arg Lys Phe Pro Arg Ala Met Pro Ile Trp Ala Arg Pro Asp
305                 310                 315                 320

Tyr Asn Pro Pro Leu Leu Glu Ser Trp Lys Asp Pro Asp Tyr Val Pro
                325                 330                 335

Pro Val Val His Gly Cys Pro Leu Pro Pro Ala Lys Ala Pro Pro Ile
            340                 345                 350

Pro Pro Pro Arg Arg Lys Arg Thr Val Val Leu Ser Glu Ser Thr Val
        355                 360                 365
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Ser Ser Ala Leu Ala Glu Leu Ala Thr Lys Thr Phe Gly Ser Ser Glu
370 375 380

Ser Ser Ala Val Asp Ser Gly Thr Ala Thr Ala Ser Pro Asp Gln Pro
385 390 395 400

Ser Asp Asp Gly Asp Ala Gly Ser Asp Val Glu Ser Tyr Ser Ser Met
405 410 415

Pro Pro Leu Glu Gly Glu Pro Gly Asp Pro Asp Leu Ser Asp Gly Ser
420 425 430

Trp Ser Thr Val Ser Glu Glu Ala Ser Glu Asp Val Val Cys Cys
435 440 445

<210> 19
<211> 447
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<213> Hepatitis C virus

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Ser Gly Ser Trp Leu Arg Asp Val Trp Asp Trp Ile Cys Thr Val Leu
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Thr Asp Phe Lys Thr Trp Leu Gln Ser Lys Leu Leu Pro Arg Leu Pro
20 25 30

Gly Val Pro Phe Phe Ser Cys Gln Arg Gly Tyr Lys Gly Val Trp Arg
35 40 45

Gly Asp Gly Ile Met Gln Thr Thr Cys Pro Cys Gly Ala Gln Ile Thr
50 55 60

Gly His Val Lys Asn Gly Ser Met Arg Ile Val Gly Pro Arg Thr Cys
65 70 75 80

Ser Asn Thr Trp His Gly Thr Phe Pro Ile Asn Ala Tyr Thr Thr Gly
85 90 95

Pro Cys Thr Pro Ser Pro Ala Pro Asn Tyr Ser Arg Ala Leu Trp Arg
100 105 110

Val Ala Ala Glu Glu Tyr Val Glu Val Thr Arg Val Gly Asp Phe His
115 120 125

Tyr Val Thr Gly Met Thr Thr Asp Asn Val Lys Cys Pro Cys Gln Val
130 135 140

Pro Ala Pro Glu Phe Phe Thr Glu Val Asp Gly Val Arg Leu His Arg
145 150 155 160

Tyr Ala Pro Ala Cys Lys Pro Leu Leu Arg Glu Glu Val Thr Phe Leu
165 170 175

Val Gly Leu Asn Gln Tyr Leu Val Gly Ser Gln Leu Pro Cys Glu Pro
180 185 190

Glu Pro Asp Val Ala Val Leu Thr Ser Met Leu Thr Asp Pro Ser His
195 200 205

Ile Thr Ala Glu Thr Ala Lys Arg Arg Leu Ala Arg Gly Ser Pro Pro
210 215 220

Ser Leu Ser Ser Ser Ser Ala Ser Gln Leu Ser Ala Pro Ser Leu Lys
225 230 235 240

Ala Thr Cys Thr Thr Arg His Asp Ser Pro Asp Ala Asp Leu Ile Glu
245 250 255

Ala Asn Leu Leu Trp Arg Gln Glu Met Gly Gly Asn Ile Thr Arg Val
260 265 270

Glu Ser Glu Asn Lys Val Val Ile Leu Asp Ser Phe Glu Pro Leu Gln
275 280 285

Ala Glu Glu Asp Glu Arg Glu Val Ser Val Pro Ala Glu Ile Leu Arg
290 295 300

Arg Ser Arg Lys Phe Pro Arg Ala Met Pro Ile Trp Ala Arg Pro Asp
305 310 315 320

Tyr Asn Pro Pro Leu Leu Glu Ser Trp Lys Asp Pro Asp Tyr Val Pro
325 330 335

Pro Val Val His Gly Cys Pro Leu Pro Pro Ala Lys Ala Pro Pro Ile
340 345 350

Pro Pro Pro Arg Arg Lys Arg Thr Val Val Leu Ser Glu Ser Thr Val
355 360 365

Ser Ser Ala Leu Ala Glu Leu Ala Thr Lys Thr Phe Gly Ser Ser Glu
370 375 380

Ser Ser Ala Val Asp Ser Gly Thr Ala Thr Ala Ser Pro Asp Gln Pro
385 390 395 400

Ser Asp Asp Gly Asp Ala Gly Ser Asp Val Glu Ser Tyr Ser Ser Met
405 410 415

Pro Pro Leu Glu Gly Glu Pro Gly Asp Pro Asp Leu Ser Asp Gly Ser
420 425 430

Trp Ser Thr Val Ser Glu Glu Ala Ser Glu Asp Val Val Cys Cys
435 440 445

<210> 20
<211> 447
<212> PRT
<213> Hepatitis C virus

<400> 20
Ser Gly Ser Trp Leu Arg Asp Val Trp Asp Trp Ile Cys Thr Val Leu
1 5 10 15

Thr Asp Phe Lys Thr Trp Leu Gln Ser Lys Leu Leu Pro Arg Leu Pro
20 25 30

Gly Val Pro Phe Phe Ser Cys Gln Arg Gly Tyr Lys Gly Val Trp Arg
35 40 45

Gly Asp Gly Ile Met Gln Thr Thr Cys Pro Cys Gly Ala Gln Ile Thr
50 55 60

Gly His Val Lys Asn Gly Ser Met Arg Ile Val Gly Pro Arg Thr Cys
65 70 75 80

Ser Asn Thr Trp His Gly Thr Phe Pro Ile Asn Ala Tyr Thr Thr Gly
85 90 95

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Pro Cys Thr Pro Ser Pro Ala Pro Asn Tyr Ser Arg Ala Leu Trp Arg
            100                 105                 110

Val Ala Ala Glu Glu Tyr Val Glu Val Thr Arg Val Gly Asp Phe His
        115                 120                 125

Tyr Val Thr Gly Met Thr Thr Asp Asn Val Lys Cys Pro Cys Gln Val
    130                 135                 140

Pro Ala Pro Glu Phe Phe Thr Glu Val Asp Gly Val Arg Leu His Arg
145                 150                 155                 160

Tyr Ala Pro Ala Cys Lys Pro Leu Leu Arg Glu Glu Val Thr Phe Leu
                165                 170                 175

Val Gly Leu Asn Gln Tyr Leu Val Gly Ser Gln Leu Pro Cys Glu Pro
            180                 185                 190

Glu Pro Asp Val Ala Val Leu Thr Ser Met Leu Thr Asp Pro Ser His
        195                 200                 205

Ile Thr Ala Glu Thr Ala Lys Arg Arg Leu Ala Arg Gly Ser Pro Pro
    210                 215                 220

Cys Leu Ala Ser Ser Ser Ala Ser Gln Leu Ser Ala Pro Ser Leu Lys
225                 230                 235                 240

Ala Thr Cys Thr Thr Arg His Asp Ser Pro Asp Ala Asp Leu Ile Glu
                245                 250                 255

Ala Asn Leu Leu Trp Arg Gln Glu Met Gly Gly Asn Ile Thr Arg Val
            260                 265                 270

Glu Ser Glu Asn Lys Val Val Ile Leu Asp Ser Phe Glu Pro Leu Gln
        275                 280                 285

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